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## ANIMAL-POULTRY AND PRODUCTS RESEARCH PROGRESS REPORT

### Part II of

### Animal-Poultry and Products Research

A summary of current program and preliminary report of progress of the United States Department of Agriculture and related work of the State Agricultural Experiment Stations.

This progress report is primarily a research tool for use of scientists and administrators in program coordination, development, and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of research progress include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members, and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of U.S.D.A. and cooperative research issued during the past year. Current agricultural research findings are also published in the monthly U.S.D.A. publications, Agricultural Research and The Farm Index.

UNITED STATES DEPARTMENT OF AGRICULTURE  
Washington, D. C. 20250

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The research program reported in this volume Part II, Animal-Poultry and Products Research, pertains to work applicable to more than one species of domestic farm animals and to more than one livestock product. It is a companion volume for supplementing: Part I-a, Beef Cattle; Part I-b, Dairy; Part I-c, Poultry; Part I-d, Sheep and Wool; and Part I-e, Swine.

The following subject matter progress reports are prepared by U.S.D.A. The number prefixes refer to advisory committees listed later that review the research reported:

- 6 - Forestry (other than Forest Service)
- 7 - Beef Cattle, Part I-a
- 7 - Dairy, Part I-b
- 7 - Poultry, Part I-c
- 7 - Sheep and Wool, Part I-d
- 7 - Swine, Part I-e
- 7 - Animal-Poultry and Products, Part II
- 8 - Cotton and Cottonseed
- 9 - Grain and Forage Crops
- 10 - Horticultural Crops
- 11 - Oilseed and Peanut
- 11 - Sugar
- 13 - Tobacco

The information contained in the above subject matter reports was first reported in the following organizational unit reports. As above, the number prefixes refer to advisory committees listed later that review all of the work of the respective divisions or services.

#### Agricultural Research Service (ARS)

- 1 - Agricultural Engineering
- 1 - Soil and Water Conservation
- 2 - Utilization -- Eastern
- 2 - Utilization -- Northern
- 2 - Utilization -- Southern
- 2 - Utilization -- Western
- 3 - Human Nutrition
- 3 - Clothing and Housing
- 3 - Consumer and Food Economics
- 4 - Market Quality
- 4 - Transportation and Facilities
- 7 - Animal Husbandry
- 7 - Animal Disease and Parasite
- 12 - Crops
- 12 - Entomology

#### Economic Research Service (ERS)

- 1, 5 - Economic Development
- 4, 5 - Marketing Economics
- 5 - Farm Production Economics
- 5 - Economic and Statistical Analysis
- 5 - Foreign Development and Trade
- 5 - Foreign Regional Analysis
- 5 - Natural Resource Economics
- 6 - Forest Service - Research (FS)
- 4, 5 - Farmer Cooperative Service (FCS)
- 4, 5 - Statistical Reporting Service (SRS)

The research program of the Department of Agriculture is reviewed annually by the following advisory committees:

1. Farm Resources and Facilities Research
2. Utilization Research and Development
3. Human Nutrition and Consumer Use Research
4. Marketing Research
5. Agricultural Economics Research
6. Forestry Research
7. Animal and Animal Products Research
8. Cotton Research
9. Grain and Forage Crops Research
- New* — 10. Horticultural Crops Research
11. Oilseed, Peanut and Sugar Crops Research
12. Plant Science and Entomology Research
13. Tobacco Research

A copy of any of the reports may be requested from Max Hinds, Executive Secretary, Animal and Animal Products Research Advisory Committee, Research Program Development and Evaluation Staff, U. S. Department of Agriculture, Washington, D. C. 20250





## I. FARM RESEARCH

### ANIMAL BIOLOGY

#### Animal Husbandry Research Division, ARS

Problem. The extent of applicable results in animal husbandry research is severely limited by a paucity of basic information on the genetics, nutrition, and physiology of our livestock. The basic research in progress has resulted primarily in revealing the inadequacy of our information regarding the functional processes within the animals we are trying to control and develop for human use. Basic research is required in such fields as animal cell metabolism and reproduction, enzymology, physiological bases for heredity, and microbiology of the rumen and intestines. Results of such studies provide the basis for additional research applied to the husbandry of each type of livestock.

#### USDA AND COOPERATIVE PROGRAM

This area consists of basic research conducted by geneticists, biochemists, physiologists, and nutritionists. It includes studies in the Pioneering Laboratories on somatic variations of red cell antigens, on the nature of the specificity of antigens and antibodies, on methods and theories of population genetics, and on the physiological aspects of hormones. Research in reproductive physiology is in progress to determine the biochemical composition and the defensive mechanisms of the uterus. Still other physiological investigations are involved with the response of mammary tissue to invasion by infectious agents. One study of long standing has the primary objective of describing, fully and in detail, the gross and microscopic anatomy of the domesticated fowl. Research on the metabolic role of vitamin B<sub>12</sub>, the investigation of unidentified nutrients in food and feed, and biological and chemical studies of rumen metabolism are also being undertaken. Investigations are in progress on the development of counter measures to prevent contamination of animal products by fallout from nuclear explosions. The work is conducted at Beltsville, Maryland; East Lansing, Michigan; and in cooperation with the Indiana, Iowa, Maryland, Michigan, Minnesota, New York, Utah, and Wisconsin Agricultural Experiment Stations.

The Federal scientific effort devoted to research in this area totals 26.5 professional man-years. Of this total, 6.0 are in agricultural chemicals, 5.0 in avian anatomy, 4.0 in genetics, 5.9 in physiology, 2.0 in nutrition, 2.6 in rumen function, and 1.0 in radioactive fallout.

There are 8 grants involving Public Law 480 funds in foreign countries financing research related to animal biology. Two are with the National and University Institute of Agriculture, Rehovot, Israel, and provide for (1) studies on the separation of young and old spermatozoa and (2) investigation of factors affecting long-term storage of sperm. They are supported for 3 years (1963-1966) with a total of \$86,904 equivalent in Israeli pounds.

There are 3 PL 480 projects in Poland. One is on the secretion of anterior pituitary hormones and ovulation in small ruminants. It is supported for 5 years (1960-1965) by \$52,455 equivalent in Polish zlotys at the Polish Academy of Sciences, Jablonna. A 4-year project (1962-1966) at the College of Agriculture, Poznan, is concerned with protein compounds of vitamin B<sub>12</sub> and its analogs. It is supported with \$38,138 equivalent in Polish zlotys. Another project is at the Polish Academy of Sciences, Warsaw. It involves the determination of the metabolic pathway of protein biosynthesis in the liver and has a 5-year duration with support amounting to \$60,411 equivalent in Polish zlotys.

A project entitled, "Study of metabolism of zinc in living organisms by means of zinc 65," is in progress at the Institut Espanol de Fisiologia y Bioguimica, Madrid, Spain. It has a duration of 4 years (1961-1965) and is supported by \$35,277 equivalent in Spanish pesetas.

The University of Montevideo, Uruguay, is conducting a project of 5 years' duration on the nutritional value of fish silage. It has \$112,785 equivalent in Uruguayan pesos of support.

A project was initiated with the Hebrew University, Jerusalem, Israel, on the effect of X-rays on viability genes with special reference to their action in heterozygotes and to the mechanism of heterosis. The project duration is 4 years and has support of \$34,650 equivalent in Israeli pounds.

#### PROGRAM OF STATE EXPERIMENT STATIONS

Physiological conditions associated with the female animal in the onset of puberty, estrus, pregnancy, and lactation are being studied with primary emphasis on the levels and interaction of the several hormones. Endocrine and environmental influences on egg production of birds are being investigated. Many of these studies require detailed chemical and histological determinations. Detailed efforts are also required with semen including production, evaluation, and preservation. One such study includes comparison of mature and immature spermatozoa and somatic cells with respect to nucleic acids and lipoprotein-carbohydrate complexes. A portion of this work is conducted under regional projects NC-41, Endocrine Factors Affecting Reproduction in the Bovine Female; W-49, Physiological Factors Affecting Fertility in Cattle; NCR-45, Poultry Physiology; and NCR-26, Artificial Insemination of Swine. Eleven States in the western region and the U. S. Department of Agriculture are cooperating in regional project W-46, The Effects of Environmental Stresses on Range Cattle and Sheep Production.

Laboratory animals such as mice, rats, quail, fruit flies, and flour beetles are used to develop and evaluate new animal breeding concepts for later application to domestic animals. Automatic data processing machines also provide early testing and evaluation of genetic theory. Research on gene

action makes use of genetic defects such as dwarfism in beef cattle and riboflavin deficiency in chickens. Blood group genes, histocompatibility loci, and plumage color genes are studied in relationship to economic traits. Biochemical mechanisms and cytogenetics of domestic animals are now receiving more attention. Much of this research is coordinated under NCR-21, Population Genetics.

Basic studies on nutrient metabolic pathways and related enzyme systems of poultry, swine, sheep, and dairy and beef cattle are in progress. Other aspects under investigation are nutrient interrelationships and toxic factors in feedstuffs as they affect requirements for growth, egg production, gestation, and lactation. Natural dietary chelates and their effect on trace mineral metabolism, plane of nutrition and resistance to disease, dietary effects upon maturation of digestive function in both ruminant and non-ruminant animals are being investigated. The use of laboratory animals in pilot studies for large animal nutrition, or to determine basic nutritional principles, is also included in this area. There are several cooperative regional projects (NC-63, NE-24, NCR-42, S-45, and W-34) that are considering various factors affecting the nutritive requirements of livestock and the nutritive value of feedstuff.

The State stations have 214.0 professional man-years devoted to Animal Biology.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Agricultural Chemicals

This research on basic metabolism, fate and role of agricultural chemicals ingested by livestock is conducted at the Metabolism and Radiation Research Laboratory, Fargo, North Dakota.

1. Herbicides. The metabolic fate of the triazine herbicides in the animal has received little attention, although these compounds are extensively used for weed control. Research has been initiated with propazine (2-chloro-4, 6-bis-(isopropylamino)-1,3,5-triazine) and simazine (2-chloro-4, 6-bis-(ethylamino)-1,3,5-triazine). Results indicate that the rat readily metabolizes and excretes propazine and simazine. Within two days of the administration of  $C^{14}$  ring-labeled propazine dissolved in corn oil, 85% of the ring had been excreted in the urine and feces. None of the ring activity appeared in the respiratory  $CO_2$ , indicating that no ring cleavage had taken place. The repeatability of this rate of excretion from animal to animal was very good, with 66% of the  $C^{14}$  appearing in the urine within two days. Conditioning the animal to the presence of propazine in the diet had little effect on this metabolism. Simazine, labeled with  $C^{14}$  in the ethylamino side chains, was similarly administered to the rat. The side chains were rapidly metabolized by the rat. Radioactive respiratory  $CO_2$  appeared within 30 minutes of administration. Seventy-eight percent of the activity excreted in the  $CO_2$ , urine, and feces appeared within 48 hours.



The above data indicate that the triazine ring remains intact and that the side chains are removed, possibly by oxidative deamination. It is not known, however, if the isopropylamino groups of propazine will be handled in the same manner as the ethylamino groups of simazine. If the metabolic pathway of propazine should be oxidative deamination of hydroxy-propazine, one should find cyanuric acid in the urine. No activity could be found by gas chromatography in the area where methylated cyanuric acid appears.

In another study, ring-labeled C<sup>14</sup> propazine was administered by capsule to a milk goat. Urine was sampled hourly, milk every 4 hours, and feces every 24 hours; tissues were sampled at the termination of the experiment. To date only the urine has been assayed for radioactivity. Radioactivity was detected in the urine one hour after dosing, with 42% of the activity being excreted in 72 hours. (AH kl)

2. Feed additives. Prior research has shown that the iodine of diiodosalicylic acid (DIS) used as a carrier of iodine in some mineralized salts is only partially available to the animal. Studies of the metabolism of this compound in the animal are in progress to determine if DIS interferes with the binding of thyroxine by specific plasma proteins, and to identify the metabolic products of DIS metabolism. DIS was added to fresh plasma in a range of concentrations. The plasma was subjected, in the presence of I<sup>125</sup> labeled thyroxine, to electrophoresis on cellulose acetate strips with a barbitol buffer at pH 8.6. In all cases, the radioactivity was concentrated in a band close to the alpha globulin region, and there was no effect on the binding of thyroxine by presence of DIS. DIS labeled with I<sup>125</sup> was synthesized, and added to bovine serum in vitro. After electrophoresis, the radioactivity of I<sup>125</sup> was again found in a band corresponding to the location of the albumen fraction.

A lactating dairy cow was given approximately .75 mc. of I<sup>125</sup> labeled DIS orally in a gelatin capsule, and urine, feces, and milk were analyzed for radioactivity. Estimates of radioactivity in the thyroid were made. At the end of 7 days postdosing, 75.2% of the dose had been excreted in the urine, feces, and milk. In addition, the thyroid contained approximately 4.1%. This accounted for 80% of the administered dose. Some loss of urine did occur, which could account for an additional but unknown amount. The results suggest that the majority of DIS excreted in the urine is still in the original form ingested. Efforts are being continued to further purify the extracts in order to achieve more definite identification of the urinary excretion products. (AH kl)

3. Microbiological studies. Microbiological studies have been initiated to investigate the role of the rumen microorganisms in the metabolic fate of agricultural chemicals in the ruminant animal, and the physiological and metabolic effects these chemicals have on the rumen flora. Considerable effort has been devoted to developing and establishing techniques and methodology for the in vitro screening of the extent of ruminal bacteria



metabolism of agricultural chemicals. Some 42 pesticides, insecticides, and herbicides were incubated with rumen bacterial suspensions for 50 to 80 minutes without showing significant endogenous gaseous increases. Thus the chemicals did not affect the metabolism of the microbes. Furthermore, Trifluralin, Amitrole, and Tabutrex were not utilized as a carbon source for growth in synthetic or complex mediums devoid of carbohydrates.

A 10-day continuous culture experiment was conducted in an effort to adapt a mixed rumen bacterial population to Amitrole in a 0.1% (w/v) concentration. The culture medium during this experiment was collected, freeze dried, extracted, and eluted on cation exchange resin columns. Thin layer chromatography of the eluted materials indicated that no metabolites were formed. Also, three consecutive 24-hr. serial inoculations were made into fresh medium containing Amitrole. The final inoculation was dispensed in  $C^{14}$ -Amitrole and allowed to incubate for 72 hours. No  $C^{14}$  metabolites were indicated.

Short-term experiments with millipore filtration techniques indicated that a mixed rumen-bacterial cell suspension of *E. simplex* will not take up  $C^{14}$  Amitrole. Steam distillation experiments indicated no  $C^{14}$  volatile materials present in rumen-bacterial inoculated media.

Ring-labeled  $C^{14}$  propazine and  $C^{14}$  atrazine and side-chain-labeled  $C^{14}$  simazine showed no metabolites with mixed rumen-bacterial populations when incubated in media varying in levels of organic nitrogen or carbohydrate constituents for periods of 1 week duration. Gaseous materials from these cultures were compared to the gases from a uniformly labeled  $C^{14}$  glucose culture, run in parallel with the pesticide substrates. No alterations of the pesticidal substrates were indicated with paper chromatography. (AH kl)

## B. Avian Anatomy

Continued progress has been made on the study of the integumentary system. Text and illustrations for the first chapter on the origin of birds and breeds of fowl have become so voluminous that it will be completed as a separate book. Of the remaining 11 chapters, for which chapter titles were given last year, 7 have been completed or nearly so. Approximately 400 illustrations have been completed, about 100 of which were done this year. The integument is being described for 5 domesticated species, chicken, turkey, domestic duck, Coturnix quail, and common pigeon.

In order to present at least a sample of factual data from these studies, mention will be made of the anatomy and histology of the sternal bursa. In spite of the importance of breast blisters, no publication exists on the normal structure of this organ and all terms used here are new. An incision through the skin of a normal adult chicken, in the mid-ventral line of the keel exposes a flattened superficial bursal cavity. Intrabursal connective tissue strands extend across the mid-line and attach to the dorsal surface

of the dermis. The peripheral boundary of the cavity is not clearly defined, and air foam vesicles along the perimeter may be present. The bursa may be made up of 2 instead of a single cavity. The second, or deep bursal cavity, lies parallel and dorsal to the superficial cavity. The layer separating them is a dense sheet of connective tissue, the intrabursal lamina. It may be perforated by intrabursal stoma which connect the 2 cavities. The deep cavity is not crossed by intrabursal connective tissue strands. The roof of the deep bursal cavity is a glistening sheet of dense connective tissue that forms the periosteum of the overlying keel bone.

Microscopic sections show a thick cornified epithelium and a dense dermis. The connective tissues that form the strands are often in the form of whorls. The connective tissues of the intrabursal lamina are densely packed. The connective tissue cells are flattened so that cavity surfaces are smooth.

The delicate intrabursal connective strands may become enlarged and form massive bars crossing the superficiales. The stoma may be coalesced so that the superficial and deep cavities are widely confluent. This range of morphologic variation occurs in the turkey as well as in the chicken. A sternal bursa is absent from young domesticated birds and it remains still a question whether a sternal bursa is a normal structure or develops only as a result of friction and irritation as certain atypical ones in man. For example, individuals who carry heavy loads on their shoulders may develop bursa where they are not normally present. (AH e8-1)

### C. Genetics

1. Methods and theories of population genetics. The nature and causes of selection plateaus, and methods for overcoming them, are being explored in mice. The two populations being used are Goodale's Large White Strain (G) and Falconer's NF Strain (F). Strain G has been selected for large 60-day body weight for more than 90 generations. Strain F had been selected by Dr. D. S. Falconer of Scotland for large 42-day body weight for 34 generations prior to importation. It has been selected for an additional 22 generations for large 60-day body weight since then. Analyses show that the G Strain has been plateaued for more than 50 generations. During this period, realized heritability was approximately zero, but estimates of heritability by the usual methods remained positive and appreciable. Strain F also appears to have reached a selection plateau for 60-day body weight.

Reciprocal crosses were made between Strain G and Strain F. In addition, the pure strains, F and G, were reproduced from the same selected males. Females were unselected and were assigned at random to pure or straincross matings. Strains G and F were very similar in body weight at weaning (28 days) and at 42 and 60 days of age. This was the case for both sexes, with a slight advantage in favor of Strain F. It is rather surprising to find that two strains with such different origins and selection histories



have plateaued at approximately the same level of body weight. Although average litter size at birth differed, average litter size at weaning was almost the same (6.6 offspring) in the two strains. Estimates of the heritability for body weight at 28, 42, and 60 days of age (based on the regression of male offspring on sire) were .11, .10, and .54, respectively, in Strain G; and 0, 0, and .11, respectively, in Strain F. Estimates of heritability at the same three ages, based on regression of female offspring on sire, were 0, .10, and .69, respectively, in Strain G; and 0, 0, and 0 in Strain F. The bias in heritability estimates of 60-day body weight persists in Strain G but is probably absent in Strain F. It appears that the plateau in Strain F has occurred because of the depletion or near-depletion of additive genetic variation.

The average amounts of heterosis obtained in the reciprocal straincross were 0%, 2%, and 3.2% for body weight at 28, 42, and 60 days of age, respectively. These amounts are less than expected. Litter size at birth was only 2% larger for straincrosses than for pure strains, but the difference increased to 8% for litter size at weaning. There was lower mortality in straincross than pure strain offspring. Estimates of heritability of litter size were .18 in Strain G at birth and zero at weaning, but were zero at both times in Strain F. Strain G is known to have a high secondary sex ratio (% males at weaning) and this was also observed in the straincross litters produced by Strain G females. No evidence of heritability of sex ratio was obtained.

The differences between body weights of reciprocal straincrosses were significant for each sex at each age. Offspring from the F male x G female mating were larger than those from the reciprocal cross. These differences may be due to differences in maternal effects and sex-linkage, but the excellent nursing ability of Strain G dams is probably a major factor.

Selection has been continued in the Goodale population selected for percentage of white hair, and a relaxed selection group has been established. The total number of all-white individuals has been increased to 29, and only six of these were females. The difficulty in reproducing this strain and obtaining satisfactory selection differentials has been alleviated by testing and then using a high-fat ration during mating and nursing. Large increases were obtained in litter size at weaning, weaning weight, and post-weaning survival. This should improve selection progress. No effect of the ration change on the selected trait was noted.

The cross of all-white males with females from an unselected strain has been carried through the  $F_3$  and  $F_4$  generations. The skewed distributions and low mean percentage of white persist. Inbred lines with various percentages of white hair are being started from  $F_4$  individuals.

The genetics of some biochemical traits related to growth are being studied. Intra-peritoneal injection of mice with  $C^{14}$ -labeled alpha-aminoisobutyric

acid at 27, 41, or 59 days of age was followed by slaughter 28 hours later. The chemical is a non-metabolizable amino acid. Two inbred strains with different growth rates and a cross between them were studied. Radioactivity was measured in blood plasma, liver, and muscle tissue in a liquid scintillation counter. The ratio of tissue to plasma activity provides a measure of the rate of protein metabolism. Preliminary results indicate that metabolic activities of both liver and muscle tissue exhibit individual and strain differences.

An experiment is under way which seeks to establish detailed curves (and mathematical expressions) for growth in four selected and unselected strains of mice. Body weight and tail length are measured on individuals at 3-day intervals from 3 to 60 days of age and at 6-day intervals from 60 to 96 days. Results of the first replication indicate distinct changes in growth rate in some strains and very smooth curves for tail length measurements. (AH p-2)

## 2. Basic research on blood antigens and antibodies.

(a) Somatic variation of red cell antigens. In studies of somatic variation of pigeons the anti-A agglutinin, Phaseolus lunatus, has been used. The antigen on pigeon red cells has been identified as serologically similar to, if not identical with, the A<sub>2</sub> substance of human red cells. However, the pigeon A<sub>2</sub> substance is not inherited in the apparently simple fashion of the human A<sub>2</sub> substance. Preliminary family data indicate that the amount of the A<sub>2</sub> antigen in pigeons is inherited and that there is more than one gene involved in the production of the pigeon A<sub>2</sub> substance.

A comparison has been made of the red cell agglutinin titer and the A-inagglutinable cell frequency in pigeons, as determined by an isotope dilution procedure using chromium<sup>51</sup>. A very close relationship was found between cell titer and the inagglutinable cell frequency, which suggests a high correlation between the inagglutinable cell frequency and the genotype for the A<sub>2</sub> antigen.

Studies of the radiation effect on the frequency of the A<sub>2</sub> inagglutinable cells were continued employing a dose rate of 360 r of x-rays. The results obtained were as follows:

<u>Sex</u>	<u>Treatment</u>	<u>Change in inagglutinable cell frequency after irradiation</u>	
		<u>Time After Irradiation</u>	
		<u>2 Months</u>	<u>4 Months</u>
Males	1. Irradiated	1.88 ± 0.69	3.72 ± 0.76
	2. Non-irradiated	0.27 ± 0.45	-0.70 ± 0.31
Females	1. Irradiated	3.41 ± 1.01	5.97 ± 1.77
	2. Non-irradiated	1.42 ± 0.06	-0.05 ± 0.71

The responses of both irradiated males and females, on the average, were significantly greater than their respective non-irradiated controls both at 2 months and at 4 months. They were also significantly higher than the pre-irradiation frequency. Most birds who responded poorly at 2 months showed a definite response at 4 months post-irradiation.

The apparent failure of some pigeons to respond following x-irradiation has been considered previously. Additional irradiation of these birds resulted in a decrease in the number of inagglutinable cells and negative selection was suggested as an explanation for this result. Survival of the A<sub>2</sub> inagglutinable cells was tested by injecting Cr<sup>51</sup> labeled blood back into the donor from which it was drawn and by comparing the inagglutinable cell frequency prior to labeling with that of the labeled blood drawn 7 days following transfusion. Three birds tested in this way showed a marked decrease (-5.32, -2.45, and -5.39) in the number of inagglutinable cells. In contrast the total cell population survival was normal. These results indicate that the A<sub>2</sub>-inagglutinable cells are undergoing negative selection. Results of a similar experiment conducted with birds previously exposed to x-irradiation are also indicative of selection against the A<sub>2</sub> negative cells. (AH p-1)

(b) Antibody specificity. Previous investigations have established the presence of agglutinins in human sera specific for human A<sub>2</sub> red blood cell stroma which can be differentially eluted at various temperatures. It was believed that the thermally dissociated fractions varied in size. To test this hypothesis, two types of experiment were conducted. In the first, gradient ultracentrifugation with linear gradients of sucrose between 20-39% were employed. Density gradient fractions were absorbed to ghosts and elutions carried out at 37°, 45°, and 56° C. In the heaviest fraction, 40.7% of the agglutinin was eluted at 56° whereas only 25.7% was eluted at 37°. The proportion of material eluted at 56° decreased progressively from the heavier to the lighter weight components. In contrast, the amount of serological activity eluted at 37° increased from the heaviest fraction to the lightest fraction. The ratio of activity eluted at 37° to that eluted at 56° was 0.634 and 3.21 for the heaviest and the lightest fraction, respectively.

In the second type of experiment, eluates obtained by thermal dissociation were subjected to starch gel electrophoresis. The ratio of eluates migrating to the cathode versus the anode was obtained. The ratio for the agglutinin eluted at 37° was 1.813, and at 56°, 0.055. A similar result was observed with fractions obtained by density ultracentrifugation; the ratio was 1.22 with the light components and decreased progressively to 0.29 for the heavy fractions. (AH p-1)



#### D. Physiology

##### 1. Physiological and biochemical characterization of uterine tissue.

(a) Quantitative characterization of uterine vascular permeability changes with estrogen. The uterine water and electrolyte changes occurring after estrogen administration have been thought to be due to an alteration in the permeability of the uterine capillary membranes. A quantitative method using the dye trypan blue was utilized to determine what proportion of the estrogen effects were due to increases in uterine blood volume and what proportion could be ascribed to changes in uterine permeability. At two hours after estrogen treatment, uterine blood volume had increased to a level three times that of the control. Increases in uterine permeability did not occur until four hours after the estrogen treatment. Permeability remained high at 6 and 8 hours, but by 12 hours had returned to control levels. (AH h5-8)

(b) Role of histamine in the mechanism of estrogen action in the rat uterus. Histamine has recently been proposed as being the mediator of estrogen action in the uterus, and studies were undertaken to determine whether histamine could reproduce the vascular permeability changes that estrogen produce in the uterus. Histamine caused a diphasic response in permeability, measured quantitatively by trypan blue accumulation in the uterus. A marked increase occurred at both 1/2 hour and 4 hours after histamine treatment. This type of permeability response is characteristic of tissue injury. A similar pattern is produced by burns and bacterial injection. Gross and histological examination demonstrated that histamine caused hemorrhage, stasis of blood in the uterus, and loss of the uterine epithelium. The pH of the histamine solution per se was not responsible for the uterine response. Estrogen caused an increase in uterine permeability at 4 hours but did not elicit the early 1/2 hour response. These results suggested that histamine was producing its effects primarily by causing tissue injury. (AH h5-8)

(c) Mechanism of estrogen action in stimulating glycogen synthesis in the uterus. One of the early metabolic changes induced in the uterus by estrogen is an increase in glycogen. While the mechanism is unknown, it has recently been proposed that the primary step in estrogen action depends upon a stimulation of some protein synthetic process. The present studies were undertaken to determine whether glycogen synthesis in the uterus could proceed while protein synthesis was inhibited. Cycloheximide, an antibiotic, was used to inhibit protein synthesis. It was found that cycloheximide itself was capable of stimulating an increase in uterine glycogen. Doses of 0.25 to 1.0 mg. of cycloheximide resulted in a 60% increase in glycogen in 4-1/2 hours and a 100% increase in 15-19 hours. Blood and uterine glucose levels were elevated and uterine water and weight increased following treatment. These observations suggest that the increase in the availability of the substrate, glucose, in the uterus stimulated glycogen synthesis. (AH h5-8)

2. Growth and development of mammary gland tissue. The role of the maternal placenta in mammary gland growth was determined. The presence of deciduoma, an artificial maternal placenta, induced mammary gland development at a level almost as high as in pregnant animals. Suspensions of either maternal, fetal, or whole placentae were injected into rats with various endocrine levels. These suspensions failed to cause increases in mammary gland growth above the levels already present in the recipient rats. The degree of mammary development achieved normally by pregnant rats at the end of gestation has not been attained by any of the combinations of endocrine levels and placental injections tested. (AH h5-1)

3. Inhibition of fertility by mechanical devices in the uterus.

(a) Plastic coils in ewes. A polyethylene plastic coil (pre-insertion diameter = 15 mm., length = 20 mm.) was inserted surgically into one uterine horn of each of 26 mature ewes at the Wisconsin Agricultural Experiment Station. The insertions were made on day-4 of the estrous cycle. The side of insertion was assigned randomly. On day-3, after the 1<sup>st</sup> or 2<sup>nd</sup> postoperative estrus, the new corpora lutea (CL) were marked with India ink and on day-6 the ewes were autopsied. The CL on the operative side were smaller ( $P < 0.01$  for both the unilaterally and bilaterally ovulating groups) and often were more firm and pale than those on the nonoperative side. The observed length of the operative estrous cycle was shorter for the ewes which had ovulated on only the operative side than for those which had ovulated on only the nonoperative side. These data indicate that a plastic coil inserted in one uterine horn of the ewe results in a unilateral "inhibitory" influence on the CL. (AH h5-6)

At Beltsville, ewes with plastic spirals in the uteri were mated 2 to 4 weeks postsurgery and killed 3 days later. Eleven ova recovered from 11 control ewes were cleaving normally and contained numerous accessory spermatozoa. Sixteen ova were recovered from 16 experimental ewes. None was cleaving and no spermatozoa were seen in the zona pellucida. Thirteen of the 16 ewes had a spiral in 1 uterine horn only, and 5 uncleaved ova were recovered from oviducts opposite the uterine horn containing the spiral.

There was no evidence of infection in uterine lumens of experimental ewes. Ovulation, as determined by laparotomy, occurred at the proper time, between 20 and 30 hours after the onset of estrus. Ova were apparently fertilizable, since 2 ova transferred soon after ovulation from unmated experimental to mated control ewes were cleaving 2 days later. Reproductive tracts of 6 control ewes and 5 ewes with a spiral only in 1 uterine horn were flushed with saline solution 4-24 hours postmating. Between 2,800 and 142,000 sperm were counted in flushings from both oviducts of 5 of the 6 control ewes, but no sperm were found in flushings from either oviduct of experimental ewes. Uterine horns of experimental ewes contained relatively few sperm. The intrauterine spiral apparently interfered with the mechanisms involved in sperm transport, and this probably explains the failure of ovum fertilization. (AH h5-8)



(b) Plastic coils in cattle. Plastic coils were inserted into one or both uterine horns of parous Holstein cows. The cows were bred naturally or by artificial insemination at subsequent estrous periods. When plastic devices were present in both uterine horns, conception was prevented regardless of the method of breeding, as evidenced by more than 50 infertile estrous cycles with no pregnancies. It was observed that at 3 days post-breeding, 4 of 5 ova were fertilized after natural mating, but only 1 of 6 ova after artificial insemination. In addition, 6 cows with no coils in the uterus and 3 with a coil only in the uterine horn opposite the side of ovulation were fertilized by artificial insemination. Results to date suggest that intrauterine coils interfere more with ovum fertilization if cows are bred artificially than if bred naturally. Also, it appears that interference with fertilization in artificially bred cows occurs only if a coil is present on the side of ovulation. Five cows with a coil in one uterine horn were bred naturally when ovulation was to occur on the side opposite the coil. All cows were pregnant when killed at 60-70 days post-breeding, indicating that uteri with a coil in one horn were capable of supporting a pregnancy and that the intrauterine coil did not interfere with maintenance of the corpus luteum. (AH h5-8)

#### 4. Hormonal control of the uterus.

(a) Porosity of the sheep endometrium. Endometrial vascular porosity was measured by the amount of intravenously injected trypan blue dye extracted from endometrium. Porosity was high in estrous ewes, intermediate in luteal-phase ewes, and low in ovariectomized ewes. The vascular response to histamine, injected into the uterine lumen, was greater in ovariectomized ewes than in estrous or luteal-phase ewes. It was also greater in ovariectomized ewes injected with progesterone than in those injected with estradiol or both hormones. This suggests an estrogen-dependent vasostabilizing mechanism in the endometrium. (AH h5-8)

(b) Neutrophilia in the rabbit. Effects of glucocorticoids on numbers and types of circulating leukocytes were investigated as a possible basis for intensification of induced leukocytic responses in the rabbit uterus. HCA was injected into rabbits and blood samples were taken for total and differential leucocyte counts. HCA injections were begun after blood samples were taken on the second day and continued daily for 3 days. HCA injections caused neutrophilia, lymphopenia, and eosinopenia. The neutrophilia may explain HCA enhancement of the leukocytic response to induced uterine infection. (AH h5-8)

(c) Leukocytic emigration in the rabbit. Rabbits were injected intramuscularly with hydrocortisone acetate over 2 to 4 days before inoculation of the uterine lumen with Escherichia coli. The corticoid caused a marked and consistent enhancement of the induced leukocytic response. The rabbits were injected intravenously with trypan blue 20 minutes before autopsy. Endometrial vascular permeability was measured by the amount of



dye extracted from the endometrium. The marked increase in endometrial vascular permeability that occurs during acute inflammation in ovariectomized rabbit uteri was not inhibited by the corticoids. As a further study, hydrocortisone was injected directly into the uterine lumen. It prevented the vascular response to bacterial infection observed in control rabbits, and to the corticoid suspension vehicle. (AH h5-8)

#### E. Nutrition

1. ~~B<sub>12</sub> synthesis in the rumen.~~ Two experiments measuring variations in the ~~rumen synthesis of vitamin B<sub>12</sub>~~ and its analogs have been conducted. In the first experiment, comparisons were made between the rumen contents of cows fed one of four diets, consisting of (1) chopped hay, (2) finely ground pelleted hay, (3) a hay and grain mixture, and (4) silage. In the second experiment, the cows were fed silage or hay, either ad libitum or restricted to maintenance level. The samples were analyzed by three methods, two of which measure both animal-active B<sub>12</sub> and microbiologically-active analogs, and the third of which measures only the animal-active form.

Both experiments indicate that the B<sub>12</sub> activity in the rumens of cows fed silage was 1.5 to 3 times that of cows fed chopped hay. The B<sub>12</sub> activity of cows fed hay-grain was decidedly lower than for any other group, while the activity from cows fed pellets was not greatly different from those fed chopped hay. Differences in animal-active vitamin B<sub>12</sub> accompanied differences in total vitamin B<sub>12</sub> activity. The vitamin B<sub>12</sub> activity was not significantly different between ad libitum and restricted feeding.

The vitamin B<sub>12</sub> potencies of the ingredients of the rations used in these experiments were markedly lower than the potencies of the rumen contents, and apparently could not account for the B<sub>12</sub> activity of the rumen contents. The lowest ratios between rumen contents and dietary ingredients occurred with the silage-fed cows but a marked increase in potency of the silage from the first experiment to the second was not accompanied by an increase in the B<sub>12</sub> potency of the rumen contents. The distribution between total and animal-active vitamin B<sub>12</sub> in the dietary ingredients resembled that found in the rumen contents only in the case of silage.

In studies with rats at Beltsville, results showed that several of the fatty acids produced in and absorbed from the rumen require vitamin B<sub>12</sub> for their metabolism, while others do not. Evidence was obtained indicating that vitamin B<sub>12</sub> is concerned in the metabolism of formic acid, which is produced in the rumen and is found there under certain conditions. Preliminary results appear to be consistent with the view that vitamin B<sub>12</sub> is involved in the metabolism of folic acid, which in turn is known to be concerned directly in the metabolism of formate. (AH h4-3)

2. B<sub>12</sub> in animal tissues. The concentrations of vitamin B<sub>12</sub> were determined in certain tissues of littermate rats fed a basal ration very deficient in vitamin B<sub>12</sub> or the basal supplemented with B<sub>12</sub>. The tissues of the vitamin B<sub>12</sub>-supplemented rats were 3.4 to 58.2 times higher in B<sub>12</sub> than those of the B<sub>12</sub>-deficient rats. The kidneys showed the greatest response to vitamin B<sub>12</sub> supplementation. The testes likewise showed a fairly high increase. For most other tissues, the order of increase varied widely from one group to the other. The results support the concept of the kidney as the primary organ of storage of vitamin B<sub>12</sub>, particularly of excess B<sub>12</sub>.

Next to the kidney, the adrenals and the pituitary were the highest in vitamin B<sub>12</sub> concentration, followed by the heart. The other tissues examined were generally much lower in vitamin B<sub>12</sub> with the testes and seminal vesicles usually containing the smallest amounts. (AH h4-3)

3. Protein in B<sub>12</sub>-deficient rations. Tests with rats showed that commercial isolated soy protein, supplemented with methionine, serves as a satisfactory source of protein in a vitamin B<sub>12</sub>-deficient ration. Results were not significantly different from those obtained with Beltsville 10x-hot-alcohol-extracted casein. Without supplemental methionine, soy protein was inadequate for normal growth even when ample B<sub>12</sub> was present. A commercial "vitamin-free" casein contained significantly more vitamin B<sub>12</sub> than did Beltsville-extracted casein. (AH h4-3)

4. Protein source in A-deficient rations. Tests were conducted with laboratory rats to obtain information on whether a commercially available soy protein is equivalent to casein as the source of protein in vitamin A-deficient rations.

The soy protein, supplemented with methionine, and a specially prepared vitamin A-free casein were fed at equivalent levels. They were used as the sole source of protein in purified rations containing adequate amounts of all other known nutrients except that no vitamin A was added. The soy protein was found to be fully as deficient in vitamin A as the casein. When adequate amounts of vitamin A were added to the rations, the two proteins were equal in supporting maximal growth rate in rats. (AH h4-3)

5. Unidentified nutrients. Tests indicated that a depression in growth occurs in normal rats when their ration contains either sulfasuxidine or sulfathalidine. This depression in growth can be counteracted by feeding a substance such as fishmeal. Dried skim milk, dried yeast or soy protein are similarly effective. The depression in growth on the sulfa-containing ration persists for at least 8 weeks, but does not increase after the third week on the ration.

The sulfa drug, sulfathiozole, which is absorbed much more readily than the others tested, was also used. The growth depression was markedly greater and considerable mortality ensued. Fishmeal did not affect this condition but dried liver afforded considerable protection.

David et al. (J. Nutrition, 76:223, 1962) reported that an unidentified growth-promoting factor for chicks occurs in milk products, such as dried skim milk, whey and analyzed reagent grade lactose hydrate; lactose itself was thought not to be the active agent. In continuing studies, two separate experiments were conducted in an effort to ascertain whether an unidentified growth-promoting factor found in milk products was required by rats. With rats of the strain used and under the conditions of the experiment, no evidence was found that such a factor is required by the rat. (AH h4-1)

#### F. Rumen Function

1. Methanogenic bacteria. Work done this year has concentrated upon identifying a water soluble, rumen-fluid growth factor previously recognized as required for growth of Methanobacterium ruminantium. Objectives were to find efficient methods of purifying the active material and to attempt to characterize it. Many compounds known to be active as microbial growth factors were tested analytically for their ability to replace the rumen fluid factor. None of the compounds were successful replacements. Evidence suggests that none of the presently known growth factors for micro-organisms will replace the activity present in rumen fluid and that the compound will prove an unusual requirement.

Several strains of methanogenic bacteria have been newly isolated from the rumen. One strain, in particular, appears to be similar to the M. ruminantium strain in use. It does not require the rumen fluid growth factor and should prove useful to workers studying the biochemistry of methane production. (AH h2-3)

2. Electron transport and fermentation in rumen bacteria. Work during the year confirmed previous work in demonstrating porphobilinogen synthesis from delta-aminolevulinic acid by resting cells of Bacteroides ruminicola strain GA33. Porphyrin synthesis could not be demonstrated in resting cells; growing cells being required. Also confirmed was that the tetrapyrrole requirement of B. ruminicola strains results from lack or inactivity of enzymes synthesizing the tetrapyrrole nucleus.

Cytochromes of the b type were detected in Selenomonas ruminantium strains and in a strain of Butyrivibrio fibrisolvens and Bacteroides succinogenes. They behaved similarly to the cytochromes in B. ruminicola, being reduced by glucose and oxidized by oxalacetate, malate, fumarate, and CO<sub>2</sub>, except for the cytochrome of B. succinogenes which was reduced by CO<sub>2</sub>. A similarity in electron transport systems in these organisms, with the probable



exception of B. succinogenes, is suggested. The presence of a quinone, vitamin K<sub>2</sub>, was demonstrated for the first time in rumen bacteria, in strains of B. ruminicola, S. ruminantium, and B. fibrisolvens. Neither cytochromes nor flavorproteins could be detected in a strain of Ruminococcus albus, suggesting that the electron transport system in this organism may differ from that of the other bacteria studied.

The presence of enzymes of the Embden-Meyerhof pathway of glucose degradation were demonstrated in strains of B. ruminicola and a strain of R. albus. No enzymes of the glucose monophosphate shunt pathway could be demonstrated, suggesting that it is not important in rumen bacteria. This agrees with indications by other workers that the shunt may not be of importance in the rumen fermentation. (AH h2-3)

3. Peptide utilization by Bacteroides ruminicola. Most strains of B. ruminicola can utilize peptide nitrogen but not free amino acid nitrogen for growth. The inability of B. ruminicola to take up free C<sup>14</sup> proline was confirmed, and its inability to take up significant quantities of free C<sup>14</sup> glutamic acid and free C<sup>14</sup> valine was established. Studies on the kinetics of uptake of C<sup>14</sup> labelled peptides by cells actively growing and synthesizing protein showed that non-protein-fixed C<sup>14</sup> in the cells reached concentrations as much as 300 times, thus suggesting that the uptake process is energy dependent. Similar studies of cells whose protein synthesis was inhibited by chloramphenicol showed that the C<sup>14</sup> which was concentrated in the cell leaked rapidly. This suggests that rapid intracellular hydrolysis of peptides occurs and that free amino acids cannot be held in the cell unless fixed into protein. Analysis of cell supernatants showed that the kinetics of release of free C<sup>14</sup> proline from peptides was consistent with this hypothesis. Data showing that the fixation of C<sup>14</sup> increases as peptide size increases is also consistent with the hypothesis. The question of whether or not enzymes are involved in uptake is crucial and investigations will continue.

The possibility that extracellular processes are occurring in competition with intracellular processes for peptides has been investigated. Preliminary results failed to show the presence of extracellular peptidases or deaminases. (AH h2-3)

#### G. Radioactive Fallout

1. Whole-body irradiation. At Cornell, studies on the effects of whole-body irradiation on farm animals have been completed, except for final analysis of the data. Some of the more significant physiological and pathological changes that occurred following irradiation were noted. Cows, goats, and sheep which were irradiated at 800 r or greater, died within three weeks after irradiation. In contrast, those irradiated at lower levels survived, and appeared generally healthy for 6 months after irradiation. Body temperature rose about 12 hours following irradiation

but soon returned to normal. Marked reduction in leukocytes occurred within 2 days. Gradual recovery began about 1 month later, but complete recovery did not occur within 6 months. Feed consumption and milk production generally decreased after irradiation but soon returned to the normal levels. The concentrations of the nuclides in milk usually increased for a period coinciding with decreased milk production. (AH h2-10)

2. Factors affecting strontium excretion in milk. Estimates of some of the important factors affecting strontium excretion in milk were made by utilizing results from cooperative studies at Minnesota, Iowa, and Utah. Under a stable intake of contaminated feed, the ratio of strontium to calcium in the milk is 0.1 times the ratio of strontium to calcium in the diet. The concentration of radiostrontium in milk declines by one-half in about 2-1/2 to 4 days after changing cows from a contaminated to an uncontaminated diet. On the other hand, when changing from an uncontaminated to a contaminated diet, the concentration of radiostrontium increases rapidly and reaches an essentially constant concentration in 5 to 6 days. These results are in close agreement with the laboratory tracer studies reported by other workers. The results were used in developing the background material for the Federal Radiation Council Report No. 7. (AH h2-10)

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**FUR ANIMAL HUSBANDRY**  
**Animal Husbandry Research Division, ARS**

Problem. Fur animal investigations are needed to obtain fundamental information on methods of increasing the productivity of ranch-raised fur animals, including rabbits. Controlled research is needed on the development of superior lines, or possibly new breeds, for producing higher quality fur and better rabbit meat. The genetics of mutations of mink and foxes and the inheritance of factors for quality of fur and of meat in rabbits require continuous study. Feeding investigations are needed to determine nutritive requirement of various species and the most economical sources of feed to meet their requirements. Of special need is the finding of satisfactory substitutes for expensive raw meat. Low cost byproducts of the meat and fishing industries must be under constant study to develop practical diets. Successful husbandry of these animals requires extensive study of the peculiar characteristics of reproduction and their relation to productivity.

**USDA AND COOPERATIVE PROGRAM**

This is a continuing program and involves (1) genetic investigations of traits for use in improvement of rabbits, minks, martens, and foxes; (2) research on the reproductive performance of mink, including the effects of hormones and the process of lactation; (3) estimates of genetic parameters and maternal effects concerning economic traits in the production of fryer rabbits; (4) studies with regard to the priming process in fur bearing animals through investigations of the mechanisms involved in the growth of hair follicles; (5) research on the basic nutrient requirements and nutrient utilization by mink and the development of diets based on fish, meat and their byproducts for mink, fox, and marten; and (6) the relationship of nutrient factors and physical characteristics of the diet to rabbit production, including the study of various proteins.

The work is in progress at Beltsville, Maryland; Fontana, California; Ithaca, New York; and Petersburg, Alaska. Cooperation is maintained with Swarthmore College and State Experiment Stations of Alaska, California, New York, and Wisconsin. Close cooperation is maintained with the National Board of Fur Farm Organizations.

The Federal scientific effort devoted to the research in this area totals 5.0 professional man-years. Of this number 0.5 are devoted to fur animal breeding, 0.8 to fur animal physiology, and 3.7 to fur animal nutrition and management.



## PROGRAM OF STATE EXPERIMENT STATIONS

Six State experiment stations, Alaska, California, Florida, Michigan, New York, and Oregon are conducting fur animal nutrition research, some of which is in cooperation with the U. S. Department of Agriculture. These studies are primarily concerned with establishment of basic nutrient requirements of rabbits and mink.

The value of antibiotics and antioxidants in mink diets is also being investigated. Consideration is given to development of low cost rations for mink including dry diets. The nature, cause, and control of fur abnormalities in mink are being studied. All fur animal breeding research is conducted in cooperation with the U. S. Department of Agriculture.

The total effort on fur animal husbandry at the State experiment stations is 7.5 professional man-years.

## PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Fur Animal Breeding

1. Breed and strain crossing for production of fryer rabbits. Data from Fontana processed at Davis, in cooperation with the California Agricultural Experiment Station, include that from 1543 rabbits born alive from 213 litters. In the Californian X New Zealand White crosses, crossbred rabbits with straightbred mothers averaged 6.3 rabbits born alive per litter, 71% weaned and 3.8 pounds individual weaning weight compared with 6.4, 67%, and 4.0 pounds for straightbred rabbits with straightbred mothers. Comparable results of backcross rabbits with crossbred mothers were 7.6, 80%, and 4.0 pounds, respectively. A heterotic effect from the crossbred mother was evident. (AH fl-1)

2. Genetic and other factors affecting disease resistance in suckling rabbits. Data at Fontana for the period 1948-1961 were divided into two subperiods, August 1946 to August 1958 (period 1) and September 1958 to September 1961 (period 2). Each contained about equal numbers of observations. Mortality rate and age distribution of deaths from enteritis were similar for each period. However, there was a significant increase in death rate subsequent to 15 days of age from periods 1 to 2. Also, there was a significant increase in the death rate for pneumonia from period 1 to 2, largely due to an increase in the death rate prior to 15 days of age during period 2. Season of weaning, parity (age of doe expressed by litter sequence), fostering, and litter size, were analyzed as sources of variation in death rate. Only season of weaning had an important effect and that was on death from enteritis. This seasonal effect was entirely accounted for by a negative regression on ambient temperature. (AH fl-1)

3. Genetics of red cell antigens and serum protein polymorphisms in rabbits. Using the techniques of starch gel electrophoresis and histochemistry, three different patterns of esterases have been observed in the red cells of rabbits. The three patterns were named A, B and AB and are inherited as if controlled by a single pair of autosomal alleles  $Es^A$  and  $Es^B$ . The frequencies of the  $Es^A$  and  $Es^B$  are 0.32 and 0.68, respectively, in the New Zealand Whites and 0.06 and 0.94 respectively, in the Californian. (AH f1-1)

#### B. Fur Animal Physiology

1. Effect of breeding does at various intervals following kindling on reproductive performance. At Fontana a total of 78 does were subjected to test; 41 with a breeding interval of 10-12 days, and 37 with a breeding interval of 24-25 days following parturition. The results indicate that does subjected to these rapid breeding schedules did not perform satisfactorily. The reproductive lives of the does were shortened, they produced fewer litters per doe per year, and mortality among the young rabbits tended to be greater than in litters from does on a standard breeding schedule of from 5-6 weeks following parturition. (AH f1-6)

2. Reproduction in foxes. Reproduction was maintained at a high level on diets made up of unconsumed feed from mink. Twelve litters, born between May 13 and June 22 varied in size from 2 to 10. Seventy-four pups were born and 60 were raised to one month of age. (AH f1-3)

3. Mating systems for martens. No marten young were produced for the third consecutive year, although matings were observed each year. Positive sperm checks were obtained on four of five males. Various practices were tried, including placing one male with several females, and feeding special diets. The three wild marten added to the herd have not bred in captivity and continue to be antagonistic toward other marten. (AH f1-4)

4. Evaluation of fiber population in fur animals. Density of the follicle and fiber population is an important factor in determining the value of the pelt. At Beltsville, Maryland, the experimental biopsy gun was found to be the most valuable tool for taking the sample and the histological procedure the method of choice. (AH f4-3)

5. Barbiturate anesthesia in ranch mink. Physiological experimentation with mink often requires the use of anesthetic agents. Usual practice involves using drugs and dosages recommended for dogs. A study was conducted on 160 ranch mink at Ithaca to investigate the efficacy and possible toxicity of thiamylal sodium and pentobarbital sodium as anesthetic agents, and on 30 mink to determine the efficacy of analeptic, bemigrade, in reducing the recovery time from pentobarbital narcosis. Thiamylal sodium administered intramuscularly is not a suitable anesthetic agent for use in mink. Pentobarbital sodium administered intraperitoneally at a level of 35 or 40 mg/kg. induced

light anesthesia in over 88% of mink and deep anesthesia in 75% without mortality. The protracted recovery period following 45 mg/kg. intraperitoneally could be effectively shortened by a 40 mg/kg. intraperitoneally of bemigrade 45 minutes after the injection of the barbiturate. (AH f2-1)

6. Effect of light on reproduction of mink. Studies on the effect of additional length of day during breeding and gestation through implantation were carried out at Ithaca involving 800 adult and over 2500 young mink. Two hours of additional length of day were given to males and females starting approximately two weeks before breeding and continued until after implantation of kits took place. The average length of gestation was 55.7 days in controls compared to 51.3 days in the lighted mink. While these results are very promising, further investigations must be conducted before practical recommendations to commercial mink ranchers can be made. (AH f2-1)

7. Endocrine effects on pelage cycles in mink. The pituitary gland is required in the mink for normal pelage cycles as determined by a series of experiments with hypophysectomized animals at Madison, Wisconsin. Gonadectomy and thyroidectomy failed to affect pelage cycles. Administration of pregnant mare serum, lutenizing hormone, follicle stimulating hormone, unfractionated ovine gonadotropin, and thyroid powder to intact mink failed to alter normal pelage cycles. Hydrocortisone inhibited normal hair change. A wild mustelid, the short-tailed weasel (Mustela erminea bangsi) gave essentially the same response as mink to the above treatments. (AH f2-1)

### C. Fur Animal Nutrition and Management

1. Development of mink diets based on sea fish and sea mammals. Diets containing 75% fish were compared with diets in which 10% fur seal meat replaced a like amount of fish and with diets in which the antioxidant, BHT and/or Ferrous Fumarate were added singly and in combination. Production varied from an average of 3.4 kits per female on the high fish plus BHT diet to 4.6 in the lot receiving fish, seal meat, BHT, and Ferrous Fumarate. Ferrous Fumarate effectively prevented the cotton pelt (anemic) condition and was beneficial to production. Thirteen percent more young were produced from the four lots which received this product. A level of 112 gms. of BHT per ton prevented steatitis but half that amount failed to stop heavy losses on high fish diets. There were no losses from steatitis where seal meat was fed but up to 50% of the animals were affected on fish and meat diets without BHT. Animals fed seal meat consumed slightly less feed and had higher weaning weights than those on all fish rations but the difference was not significant. (AH f3-1)



2. Feeding fresh water scrap fish to mink. Studies of methods to obtain the maximum utilization of raw fish from the Great Lakes (trawler chubs and alewives) were continued during the growth period of 1964 and the reproductive season of 1965. During the growth period 100 dark females and 90 dark males were allocated to 5 groups receiving diets of (1) ocean fish, (2) 30% alewife presscakes, (3) 30% cooked alewife, (4) 30% raw chub, and (5) 30% cooked chub. Final weights were comparable between the various groups but were less than the controls. The reproductive performance was not as satisfactory on the alewife or chub diets as in the control group. The reason for the poor performance of the mink on these diets is unknown. The possibility of agricultural or industrial contamination products, such as DDT, being present in the Great Lakes fish and not present in ocean fish is currently being investigated. (AH f3-1)

3. Digestibility of mink feeds. The determination of the protein, gross energy and dry matter digestibility of feeds available for use in mink diets is of great practical and economic value to the mink rancher. Digestibility studies were conducted on cattle and horse muscle and viscera products, poultry byproducts and viscera, several species of fish, dairy products, and desiccated live protein feeds. Several products not previously considered as good diet ingredients gave evidence of being desirable, especially from the standpoint of protein digestibility. (AH f3-5)

4. Protein requirements of rabbits. To further evaluate the protein requirements of rabbits for meat production, an experiment was conducted utilizing five diets analyzing 17.1, 17.5, 18.3, 19.4, and 20.9% crude protein on an "as fed" basis. Criteria analyzed were: number of young weaned per litter, mean weight per rabbit weaned, kilograms feed consumed per rabbit weaned, and kilograms feed per kilogram of weight weaned. No significant differences were evident among the protein levels for any of the criteria. It appeared that the crude protein requirement was met at the lowest level of 17.1% which would correspond to 19% on a dry matter basis. (AH f3-2)

5. Effect of nitrogen fertilizer on the nutritive quality of Bermudagrass. This investigation was conducted in cooperation with the University of California at Riverside. Ammonium nitrate fertilizer (32.5% N) was applied to Bermudagrass pastures at the ratio of 0, 100, and 250% N per acre on a plot laid out in three replications. For treatment diets, alfalfa hay was replaced by Coastal Bermudagrass hay fertilized at the three levels. The results indicate that diets containing fertilized Bermudagrass contained levels of nitrogen nitrate that appeared to be toxic to rabbits, and which may interfere with availability and utilization of vitamin A. The results thus suggest that rabbits may be quite sensitive to high nitrate levels, and their use in evaluating the supply of metabolizable energy in forage crops or for obtaining data on feed conversions should be carefully examined. (AH f3-2)

6. Distillers grains and solubles as plant protein supplements in the rabbit diet. Distillers dried grains and solubles when used as the only supplemental sources of plant protein in rabbit diets, produced no beneficial effects on number of young weaned per litter, weaning weight, or palatability. Conflicting results were obtained with respect to feed efficiency and number of viable young at birth. Distillers grains and solubles were not satisfactory replacements for soybean meal as the sole supplemental plant protein source. However, the addition of 10 or 30% distillers grains as replacement for part of the grain mixture gave satisfactory results. (AH f3-4)

7. Supplemental vitamin E in rabbit diets. Does were randomly assigned to three groups, receiving a control diet (Group 1), the control diet supplemented with 10,000 units of vitamin E per ton (Group 2), and the control diet supplemented with 20,000 units of vitamin E per ton (Group 3). The does were maintained on these diets through several successive litters. On the basis of the results it appears that supplemental vitamin E increased the average number of live young born per doe which might be explained on the basis of an intrauterine effect, reduced the average number of young born dead, increased the number of young weaned per doe, had no effect on individual weaning weights of the young, reduced doe mortality, and reduced mortality of the young between the ages of 22 - 56 days when the young are out of the nest box and consuming the pelleted feed. Therefore, it seems that although individual weaning weights were not increased, total production per doe per year might be increased with the use of supplemental vitamin E. (AH f3-2)

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DISEASES AND PARASITES OF HORSES  
Animal Disease and Parasite Research Division, ARS

Problem. Currently there are about 3,250,000 horses in the United States, valued at approximately \$860 million. About one million of these are draft animals. Considerable numbers of horses and mules are still required for work on cattle ranches and as pack animals. The annual overall value of the horse industry has been estimated at about \$1.5 billion. The horse may be an important link in epizootiology of animal diseases in general. Equine piroplasmosis is an acute, subacute, or chronic tick-borne disease of horses caused by protozoan parasites that was first recognized in this country in Florida in 1961. It is characterized by high fever, progressive anemia, jaundice, edema, extreme weakness and depression. Fatalities range from 5 to 50 percent of infected animals. This disease, now apparently well established in Florida, has extended into Georgia and poses a serious threat to the entire equine population in the southern United States. The disease is clinically indistinguishable from equine infectious anemia. Horses which have clinically recovered from piroplasmosis usually remain carriers of the disease and are a potential source of infection. African horsesickness, a highly fatal disease of equines, that was confined to Africa has caused serious losses in the Middle East and parts of Asia in recent years.

USDA AND COOPERATIVE PROGRAM

The Department has a program involving biochemists, pathologists, protozoologists, and veterinarians working on equine piroplasmosis. In order to be prepared in the event of introduction of African horsesickness into the United States, the Plum Island Animal Disease Laboratory has obtained African horsesickness viruses and antiserums from South Africa. These materials are thus directly available for diagnostic and vaccine studies should the need arise.

The Federal scientific effort devoted to research in this area is 2.0 professional man-years. This effort is divided among sub-headings as follows:

Serological diagnosis, transmission, and control of equine piroplasmosis 2.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland (In cooperation with the Entomology Research Division), and under contracts with the University of Florida, Gainesville, and the University of Kentucky, Lexington.

PL 480 funds have been made available in Turkey for research on *Gastrophilus pseudo-hemorrhoidalis* (equine parasite) in Turkey, and for the study of the horsesickness virus.

## PROGRAM OF STATE EXPERIMENT STATIONS

Workers in many states are continuing to develop new useful knowledge concerning the treatment and control of equine disease conditions.

Efforts are being made to study the anatomy and mechanics of bones, joints, ligaments and tendons in relation to the occurrence of lameness and certain skeletal anomalies. Some of these basic studies complement and extend knowledge concerning athletic injuries in man and skeletal problems in all animals.

Vaccines for the prevention of viral rhinopneumonitis, equine viral arteritis and influenza are being evaluated under laboratory and field conditions. Studies are continuing on the development of procedures for the diagnosis and prevention of respiratory diseases of horses.

Much effort is being made to develop and evaluate more effective drugs and procedures for the prevention and control of equine parasites. Parasite-free foals are being employed in gaining new knowledge concerning the disease-producing mechanism produced by certain internal parasites.

In addition, attention is being given to other conditions such as piroplasmosis, colitis, infertility, and diseases of the newborn.

The total State scientific effort devoted to equine disease research is 14.5 professional man-years annually.

## PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Equine Piroplasmosis

At the Beltsville Parasitological Laboratory, investigations on equine piroplasmosis have been continued in the areas of research on characterization and transmission of the protozoa Babesia caballi that causes the disease, and the development of a standard antigen for use in serological diagnostic tests. The existence in the United States of Babesia equi, another etiologic protozoan agent for equine piroplasmosis, was positively confirmed.

(BPL)

Research studies are being conducted at the Agricultural Experiment Station, University of Florida at Gainesville, under a contract with the USDA on investigations designed to evaluate chemotherapeutic methods of prevention, treatment and eradication of piroplasmosis in horses. To date a total of 132 cases have been treated, 41 of these being field cases. Nine drugs have been used and three, Berenil, Diampron, and Phenamidine, were useful in elimination of the "carrier" state.

(Florida)



Studies are under way at the Agricultural Experiment Station, University of Kentucky at Lexington, under a contract with the USDA, on investigations designed to develop antigenic material for use in diagnostic tests for equine piroplasmosis. (Kentucky) (ADP b6-13C)

B. Gastrophilus pseudoheorrhoidalis (equine parasite) in Turkey, etc.

Under a PL 480 Grant to the Veterinary Faculty, Ankara University, Ankara, Turkey, research investigations are in progress on the distribution, life cycle, treatment and control of the equine parasite Gastrophilus hemorrhoidalis. A total of 905 equines were examined for the parasite larvae and of these 675 horses and 225 donkeys were found to harbor the larvae of different Gastrophilus species. Eighty-two third instar larvae were obtained which had the characteristic body structure of the G. hemorrhoidalis. Eleven of them hatched into flies but only two reached maturity.

No destructive effect on the incubating eggs of the Gastrophilus species was observed following the application of a 1% solution of Isotox, a 0.01% solution of Asuntol, or a 10% solution of Neguvon to the skin of horses. The oral administration of Neguvon, 35 mg./kg. body weight, was found to be effective for control of second and third instar larvae of the Gastrophilus species that were not attached to the wall of the rectum. (A22-ADP-4)

C. Horse Sickness

Under a PL 480 Grant to the Veterinary Faculty, University of Ankara, Ankara, Turkey, studies of the horse sickness virus (HSV) in tissue culture, its serological and immunological characteristics, and vectors, have continued. The report shows that tissue cultures were prepared from sheep, calf, dog, donkey and goat kidney cells, and monkey cells. The horse sickness virus propagated in dog, donkey and monkey kidney cells, but not in sheep, calf and goat kidney cells. Seven virus strains were passaged 10 times and did not show any loss of capability to infect cells. Tenth passage horse sickness virus in tissue culture fluids were tested for hemagglutination with erythrocytes of guinea pig, rabbit, goat, sheep, horse, donkey, and calf. The virus showed low hemagglutination only with erythrocytes of rabbit and horse. (A22-ADP-7)

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

None

INFECTIOUS AND NON-INFECTIOUS DISEASES  
OF FUR ANIMALS INCLUDING RABBITS  
Animal Disease and Parasite Research Division, ARS

Problem. In the raising of fur animals in captivity, such as rabbits, chinchillas, mink, and foxes, disease problems incidental to the confinement of such animals are encountered. These include viral, bacterial, parasitic, mycotic, nutritional, and hereditary diseases. The enteric disease-complex causes great mortality in commercial rabbit production. It destroys whole litters and commonly attacks all susceptible rabbits on a farm. The respiratory disease-complex, perhaps, is second as a cause of mortality. In severe outbreaks over 50 percent of adult animals may die. These two diseases cause great economic loss to the rabbit industry, which produces an estimated 50 million pounds of meat annually and millions of dollars worth of rabbits for experimental purposes. Virus diseases of mink cause the greatest loss to the 7,000 mink ranchers now producing more than 5 million pelts annually valued in excess of \$100 million. The role of helminths as carriers of rickettsial and viral agents causing, or associated with diseases of fur animals, is becoming of extreme importance and is one about which little is known.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving microbiologists and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of fur animals. Research was conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 3 professional man-years. This effort was applied as follows:

Rabbit diseases 1.0 at the U. S. Rabbit Experiment Station, Fontana, California, in cooperation with the Animal Husbandry Research Division, ARS.

Coordinated Field and Laboratory Studies 1.0 at the U. S. Fur Animal Disease Research Laboratory, Pullman, Washington, in cooperation with the Washington State University.

Transmission of Infectious Diseases by Helminths 1.0 at the U. S. Fur Animal Disease Research Laboratory, Pullman, Washington, in cooperation with the Washington State University.

## PROGRAM OF STATE EXPERIMENT STATIONS

Scientists at several of the State experiment stations are conducting research on the prevention and control of diseases of fur-bearing animals.

Investigators seek to determine the cause and control of Aleutian disease in mink. Basic studies are being made concerning the transmission of the disease. The relationship of nutrition, heredity and toxins as possible causative factors is being determined. Additional studies seek to identify the etiology of enteritis in mink and to develop methods for prevention and control.

Workers also seek basic information concerning the importance and the disease-producing characteristics of pseudorabies virus in mink. Attempts are being made to provide useful information concerning the cause and control of a scrapie-like disease in mink.

A modified live-virus vaccine has been developed for the prevention of myxomatosis in rabbits. Attention is now being directed toward evaluating the vaccine and determining its effectiveness against possible other strains of the virus.

The total State scientific effort devoted to fur-bearing animal disease research is 5.7 professional man-years.

## PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Rabbit Diseases

At the U. S. Rabbit Experiment Station, Fontana, California, a survey of normal symptom-free animals indicated that 70% harbored the Pasteurella organism and 16% harbored Bordetella organisms in the nasal turbinates. The feeding of aureomycin and sulfamethazine, singly and together, had varied effects on the incidence. Aureomycin medication at 100 grams per ton of feed greatly reduced the incidence of nasal Pasteurellosis in mature animals, but had little or no effect on the fryer-size animals. Aureomycin medication also reduced the incidence of Bordetella isolations in the fryer-size animals. When aureomycin and sulfamethazine were combined, a high incidence of gastro-enteritis was encountered. Because of this high incidence of enteritis and resulting poor production, the use of this combination of drugs is not indicated in rabbit feeds.

An amylase-containing feed additive was fed to a group of animals at 2 - 4 pounds per ton levels. The addition of amylase to the ration had no effect on the incidence of enteritis. Blood amylase determinations were made on 36-day, and 7-week old fryer-sized animals. The lack of amylase in the ration does not appear to be involved with the incidence of enteritis.



Serum agglutinins produced by three commercial vaccines were studied. Agglutination levels were the highest and persisted for the longest period of time in those animals given an oil-emulsified Pasteurella vaccine.

(Fontana, California) (ADP a6-5)  
ADP a6-6)

## B. Field and Laboratory Study of the Diseases of Fur Animals

The following work has been reported from the Division's Fur Animal Disease Research Laboratory, Pullman, Washington:

Feline Cell Culture Propagated Panleukopenia Virus. Conventional neutralization tests performed in feline kidney cell cultures, using the Philips Roxane (PR) strain, and various feline virus immune sera revealed that the PR strain is either panleukopenia or a virus closely related to it. These tests also confirmed the relationship between feline panleukopenia and mink virus enteritis since both viruses stimulated the production of antibody which neutralized the Philips Roxane virus.

An antigenic extinction test using the PR virus revealed that the efficacy of cell culture fluid as a vaccine was about 300 times greater than its infectivity when measured by titrations in feline kidney cell culture.

Intranuclear inclusions were produced in cell culture. There was a relationship between the percentage of inclusion bodies and virus titer. As the titer increased, the percentage of mature inclusions also increased.

Adjuvated-Inactivated Distemper Vaccine. There was no significant difference in the ability of live or adjuvated inactivated distemper vaccines to overcome the blocking effect of maternal antibody.

Some physical and Chemical Characteristics of Partially Purified Aleutian Disease Virus. The protein content of tissue preparations can be reduced by fluorocarbon extraction without detectable effect on the biological activity of the virus. Partially purified Aleutian Disease agent showed a surprising stability against the action of proteolytic enzymes and nucleases. Conversely, it is readily inactivated by boiling or treatment with strong acids, bases or iodine. (Pullman, Washington) ADP a6-7)

## C. Transmission of Viral and Rickettsial Diseases of Helminths

At the Division's Fur Animal Disease Research Laboratory, Pullman, Washington, recent studies have demonstrated 5-1/4 years persistence of the Elokomin fluke fever agent in metacercariae of Nanophyetus salmincola recovered from steelhead trout maintained in salt water ponds. Juvenile silver salmon recovered off the coast of Alaska confirmed our previous studies demonstrating a high degree of infectivity of Pacific salmon with the vector fluke. Ten percent of these salmon, which were believed to be northbound migrants, were demonstrated to be infected with the metacercariae of

*N. salmincola*. A comparison of Elokomin fluke fever and the Sennetsu rickettsial agent of Japan is under way. (Pullman, Washington) (ADP a6-8)

## PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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MISCELLANEOUS INFECTIOUS AND NON-INFECTIOUS  
DISEASES OF ANIMALS  
Animal Disease and Parasite Research Division, ARS

Problem. Included in this area of research are studies on problems involving more than one species of domestic animal, poisoning by various plants, which differ in toxicity according to local conditions, and affect different species of animals in various ways; agricultural chemicals such as herbicides and pesticides, which may produce poisoning in animals, especially if not properly used, and may also leave dangerous residues in the soil, feed, or animal body, and bloat, a common, serious condition in cattle and sheep. Investigations of these diverse problems require modern techniques as well as fundamental approaches through chemistry, pathology, physics, physiology, and other scientific disciplines. The problems are so complex, diverse, and numerous that it has been impossible to more than scratch the surface in probing for basic knowledge required for protection of the nation's livestock and poultry populations.

USDA AND COOPERATIVE PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, pathologists, physicists, and veterinarians engaged in both basic studies and the application of known principles to the solution of miscellaneous infectious and non-infectious diseases of animals. Research is being conducted at the designated locations.

The Federal scientific effort devoted to research in this area totals 21.4 professional man-years. This effort is divided among sub-headings as follows:

Components of Normal and Immune Serum 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Bloat in Ruminants 3.3 at the National Animal Disease Laboratory, Ames, Iowa, and through cooperative agreements with the California, Maryland, Mississippi, and Wisconsin Agricultural Experiment Stations, and with the New York State Veterinary College.

Preparedness for Diagnosis of Foreign Animal Diseases 0.5 at the Plum Island Animal Disease Laboratory, Greenport, Long Island, New York.

Toxicology and Pathology Related to Insecticides 3.0 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas, in cooperation with the Entomology Research Division.

Biochemical Effects of Agricultural Chemicals 1.0 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas, and through a cooperative agreement with the Stephen F. Austin College at Nacogdoches, Texas.



Detoxication Mechanisms in Cattle and Sheep 1.0 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas.

Cytological Responses to Antiparasitic and Other Agricultural Chemicals 1.0 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas.

Poisoning by Plants 1.1 at the Logan, Utah, Field Station, through formal cooperation with the Utah Agricultural Experiment Station, and informal cooperation with the U. S. Plant, Soil and Nutrition Laboratory of the Soil and Water Conservation Service, Ithaca, New York. A PL 480 grant supports research at the Instituto Biologico, Sao Paulo, Brazil, on The Study of Plants of the State of Sao Paulo Poisonous to Domestic Animals.

Toxicity of Herbicides and Herbicide-Treated Plants for Domestic Animals 0.5 at the Logan, Utah, field station, with informal cooperation with the Utah Agricultural Experiment Station and the Crops Protection Branch of the Crops Research Division at Logan, Utah.

Biological Changes Associated with Neuropathological Conditions in Animals 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Physiopathological Investigations of the Interrelations between the Respiratory, Circulatory, and Digestive Systems of Animals 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Proteins and Other Complex Molecules from Animal Disease Agents Derived Primarily from Surface Structures and Extracellular Products 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Chemical and Physical Studies on Microbial Antigens 1.5 at the National Animal Disease Laboratory, Ames, Iowa.

Microbiology of the Ruminant Digestive Tract and Its Relation to Digestive Disturbances 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

#### PROGRAM OF STATE EXPERIMENT STATIONS

There are numerous complex problems in this area which need attention. Increased emphasis is being placed on research aimed at the toxicological and pathological effects associated with the use of insecticides and other agricultural chemicals. Efforts are being made to develop and evaluate more specific and less persistent chemicals for the control of livestock diseases. Also, considerable attention is directed toward non-chemical control measures.

A number of States are identifying and studying the pharmacological action of the toxic principals involved in major poisonous plants. Treatment measures are being continuously developed and evaluated. Other research is aimed at the toxicology and metabolic fate in animals of nitrates, urea, selenium, fluorides, lead, molybdenum, etc.

Work continues at a number of locations on the cause and prevention of bloat in ruminants. Investigations are being made concerning plant and animal interactions which contribute to the bloat problem. Preventive and treatment measures are being developed and evaluated at the same time.

Other problems and conditions receiving attention by State research scientists are various types of livestock tumors, hypertension, anesthesia, improvement in slaughter methods, neurophysiology, function studies on the hypothalamus, thyroid and adrenal glands, syndactylism, bone growth, hemodynamics and numerous physiological, neurological and anatomical studies.

The total research effort devoted to miscellaneous diseases of animals at the States is 32.7 professional man-years.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Components of Normal and Immune Serums.

At the National Animal Disease Laboratory, Ames, Iowa, changes in serum proteins were studied by paper electrophoresis for 29 weeks following exposure in midpregnancy of 18 Brucella abortus strain 19 vaccinated, and 5 nonvaccinated heifers to virulent Br. abortus strain 2308. In the serums of heifers that became infected (4 vaccinated and 4 nonvaccinated), the relative percentage of gamma globulin increased, whereas that of albumin decreased. The percentage of gamma globulin finally exceeded that of albumin. The excess of gamma globulin over albumin was greater and persisted longer in serums from infected nonvaccinated than in infected vaccinated heifers. The changes in the amount of gamma globulin roughly paralleled the changes in the serum agglutinin titers. Only minor changes occurred in the concentrations of albumin and gamma globulin in the serums from 14 vaccinated and 1 nonvaccinated heifer that did not become infected.

(NADL)

(ADP a7-14)

##### B. Bloat in Ruminants

Under a cooperative agreement with the Maryland Agricultural Experiment Station, College Park, studies designed to identify the active factor(s) in green legumes which dispose to bloat have been conducted. Alcohol extracts, saline extracts, alcohol and water extracts, and saline soluble protein have been prepared from green alfalfa. None of these preparations has given any consistent results in producing bloat in fasted sheep, in sheep fed a high concentrate bloat diet, or in sheep previously grazed on grass or ladino clover. In trials conducted during the past year, injections of atropine and adrenaline have consistently produced slight to moderate bloat in sheep on a variety of diets, and tolazoline hydrochloride and reserpine have been effective in diminishing bloat so produced.



Tyramine and/or tyrosine, administered orally to sheep injected with atropine, have not produced bloat as frequently as has the injection of epinephrine. Attempts to demonstrate atropine-adrenaline-like action in the preparations from green alfalfa have not been successful and the incidence of bloat in sheep grazed on or fed the alfalfa was quite low.

(College Park, Maryland) (ADP a7-15)

At the New York State Veterinary College, Ithaca, under a cooperative agreement, research has revealed that the transport of a fatty acid from the rumen contents to blood depends upon 1) the electrochemical gradient of the anion form, 2) the chemical gradient of the undissociated acid form, 3) the relative permeability of the epithelium to the anion and acid forms, and 4) the rate at which the fatty acid is metabolized by the epithelial tissue. Therefore, the isolated, short-circuited rumen epithelium was used to study the effects of pH, concentration gradient, imidazole vs. bicarbonate buffer systems and fatty acid metabolism on the transport of acetate propionate and butyrate. Transport was found to increase with an increase in concentration gradient or a decrease in the pH of the fatty acid solution bathing the lumen surface of the tissue, and acetate transport also was greater when the bathing solutions were buffered with bicarbonate in place of imidazole. The effect of bicarbonate buffer on acetate transport was associated with a change in short-circuit current which suggested the  $\text{HCO}_3^-$  or  $\text{CO}_2$  also affected the active transport of ions. All three fatty acids<sup>3</sup> were metabolized to a considerable extent by the tissue but their metabolism was not a simple function of the amount of fatty acid absorbed. The results, including the effect of anoxia on transport, indicated that metabolism of each of the fatty acids played a critical role in determining its rate of absorption and transport.

(Ithaca, New York) (ADP a7-15)

Researchers at the Wisconsin Agricultural Experiment Station, Madison, under a cooperative agreement, report that, in order to understand better the processes of digestion before and during the onset of bloat, experiments were carried out to determine those enzymatic activities or reactions which, by their qualitative or quantitative behavior, may influence those pathways leading to increases in the viscosity of ruminal contents. Bloat-regulating substances operating in or administered to ruminants, were also studied.

To determine which ions produced pectate gels like that of calcium, cations of zinc, aluminum, iron (ferric), copper, potassium, magnesium, manganese, and sodium were tested. Of the cations used only potassium, magnesium (sulfate), and sodium failed to form some type of gel; the other salts formed a lumpy or semi-gel. Only calcium salts formed stable pectate gels and therefore served as good models for producing a viscous rumen during bloat.

Properties of pectin methyl esterase (PME) activity in a commercial pectin esterase and in alfalfa extracts were examined and the optimal pH's, substrate and enzyme concentrations, and time of reaction were found by



titration of pectin. In addition, PME activity was found to be more inhibited at lower concentrations of the biodegradable detergent, ultrawet K soft, than by the nonbiodegradable one, K dense. The K soft surfactant has not yet been used in feeding trials of cattle during experimental bloat.

PME activity and moisture content of growing alfalfa were greater on bloating than on non-bloating days. Freezing enhanced the extraction of PME. Ruminal contents were a better PME extractor than buffered saline.

The hydroxamic acid reaction (HAR) for the presence of pectin in agar medium has been used to measure PME activity since the clear zones represented sections on the plate where the enzyme has hydrolyzed the methyl ester groups of pectin. Salts in general had very little effect on the size of clear zones but added phosphate buffer produced slightly larger zones than other salts added to acetate. The relative PME in ruminal fluids was about 0.013 percent. Larger concentrations of detergent up to 0.10% did not reduce the apparent PME activity in strained ruminal contents. The HAR method has been used successfully for a large number of experiments.

It has been shown that cellulase activity was measured by the carboxymethyl cellulose-agar pectin dish technique; cellulase was affected by pH of the substrate, time and temperature of incubation, and concentration of the carboxymethyl cellulose (CMC) in the substrate-agar system. Lower pH's, longer periods of incubation, higher temperatures and lower concentrations of CMC gave larger areas of activity. Alkyl aryl sulfonate did not inhibit cellulase activity.

In further studies with starch-degrading enzymes, it was shown primarily that centrifuged ruminal fluid from a cow bloated on pure corn and alfalfa contained 100 times more starch enzyme activity than that of non-bloated ruminal fluids.

Frothy digesta erupted from a cow resembled that of "feed-lot" bloat. The viscous nature, low pH and the yellow particles of corn entrapped in the stable foam were characteristic properties of corn or grain bloating. A large increase in starch-degrading activity and smaller increases in PME and protein-degrading activities all complement the increased viscid ruminal contents of the bloated cow. (Madison, Wisconsin) (ADP a7-15)

C. Toxicological and Pathological Effects of Insecticides, Herbicides, Fungicides, and other Agricultural Chemicals on Livestock and Poultry.

At the Division's Toxicology Laboratory, Kerrville, Texas, the following work is reported:

During FY 1965, 72 insecticides were studied in cattle, sheep, and goats, many of them as cooperative ventures with the Entomology Research Division. As in past years, the toxicities were found to run from the impossibly dangerous to reasonable safety. Of particular interest as compounds of low toxicity are ENT 25841 produced by Shell Chemical as compound 8447, and

ENT 27162 produced by Cela of Germany and sometimes called Bromophos.

Seven insecticides were studied in 280 chickens 8 to 9 weeks of age to determine the oral toxicity. The maximum nontoxic dosages found were: coumaphos, 2.5 mg./kg.; diazinon and dichlorvos, 5 mg./kg.; Ciodrin and ruelene, 100 mg./kg.; and dioxathion, 250 mg./kg.

Studies were begun in the last half of FY 1965 of insecticides employed primarily as plant treatments. Past capability permitted studies of those used directly on livestock but did not allow us to glean more than a small amount of knowledge of those used on plants. Initial studies have been with sheep to establish techniques.

One of the more interesting findings at this time is that sheep do not consume demeton (Systox), even when partially starved, when it is added to feed or sprayed on grass. This finding needs to be enlarged upon and firmly established for both cattle and sheep. If true, then poisoning of sheep and cattle would be most unlikely in a pasture where both treated and untreated forages are available.

In one study sheep were observed to increase their tolerance to malathion as both the dosage and frequency of administration were increased.

Analysis of dips revealed the depletion of each compound from the dipping fluid while wool samples from sheep in the order of their immersion demonstrated the differences in the amounts of insecticide retained on each animal.

Co-ral and one approved lindane formulation were found to maintain the original concentration throughout dipping. One toxaphene formulation was found to deplete slowly. Most other compounds and formulations depleted rapidly.

When depletion was small or nonexistent the wools of sheep received rather uniform deposits. When depletion was marked the wool deposits were highly variable.

Apholate was fed to Jersey cattle at 1 mg./kg. throughout one gestation period. A deficiency of white blood cells appeared in one heifer after 80 daily doses and in the others after 110. One heifer died after 335 doses, one delivered a calf and died after 531 doses, one delivered prematurely and survived 581 doses, and the fourth delivered at term and survived 629 doses.

Chronic feeding of apholate was continued in sheep that were also in study during FY 1964. Two rams and three ewes survived 638 daily doses at 1 mg./kg. A fourth ewe died after 574 daily doses. A moderate deficiency of white cells was developed and persisted for 128 days after the withdrawal of apholate.



Hempa appears to be of considerably less toxicity than apholate, tepa, and metepa, but shares with them the ability to produce a deficiency of white blood cells.

Twenty-one commercially available herbicides were administered orally to chickens, sheep, or cattle, in repeated daily doses. Virtually all proved to be of a low order of toxicity, indicating that the hazards of most of these compounds to domestic animals would be limited to cases of accidental spillage and similar incidents rather than the consumption of them on treated forage or feed. The study established the clinical signs and necropsy lesions to be expected in these poisonings.

Captan, Zineb and Ceresan M were studied in chickens or cattle; of the three Ceresan M is the more toxic. Cattle appear to be from two to three times more susceptible to poisoning by Ceresan M than are chickens. Massive doses of Zineb or Captan were required to produce poisoning.

(Kerrville, Texas) (ADP a7-23)

#### D. Biochemical Effects of Agricultural Chemicals and Control Substances

The following studies were reported from researchers at the Division's Toxicology Laboratory, Kerrville, Texas.

Injured or dying cells are known to leak, or discharge, enzymes into the blood stream. Certain enzymes are to be found extensively only in specific tissues such as the heart, muscles, or liver. Research during FY 1965 was devoted to determining patterns of these enzymes in average cattle then, by poisoning cattle, attempting to develop a "fingerprint" pattern of the enzymes that increased. In addition, studies were made of the effect of oxime-type cholinesterase reactivators on this "fingerprint" pattern. Results have, in general, been encouraging. The oximes appear to protect many cells from injury in addition to their key action of being antidotes for organophosphorus insecticide intoxication.

Dioxathion (Deltax). Cattle were poisoned with dioxathion to determine the effect upon certain serum enzyme systems and blood protein elements. Three oximes, 2-PAM, DAM, and TMB<sub>4</sub>, were used as antidotes at 10 and 20 mg./kg. Cattle poisoned by dioxathion show elevations in the activity of serum glutamic oxalate and pyruvate transaminases, and of alkaline phosphatase, which is indicative of tissue damage. Blood beta lipoprotein levels were elevated, while gamma globulin was decreased. 2-PAM and TMB<sub>4</sub>, when administered to cattle poisoned with dioxathion, retain the levels of the serum glutamic oxalacetic transaminase, glutamic pyruvic transaminase, and alkaline phosphatase activities at near normal. 2-PAM, at both dosage levels, lowered the level of beta lipoprotein of poisoned animals while only the higher dosage of 20 mg./kg. of TMB<sub>4</sub> did so. With all three oximes the level of gamma globulin of poisoned animals remained near normal. DAM did



not aid in reducing fatalities. At the dosages studied  $\text{TMB}_4$  appears to be slightly more beneficial than 2-PAM for dioxathion poisoned cattle insofar as biochemical effects are concerned. DAM does not appear to be beneficial at the levels studied.

Dichlorvos (Vapona). Cattle were poisoned with an oral dosage of dichlorvos to determine the effects on serum glutamic oxalacetic and pyruvic trans-aminase, aldolase and alkaline phosphatase. Oximes were given to some of the cattle to determine their protection of those enzyme systems. Severe poisoning of cattle did not follow the dosages of dichlorvos that were administered. 2-PAM and DAM retained the enzyme activities of the mildly poisoned animals near normal, whereas  $\text{TMB}_4$  appeared to cause an increase in activity above normal during the test. From the biochemical standpoint, it appears that 2-PAM and DAM offer more protection to the enzyme activities than does  $\text{TMB}_4$ .

Coumaphos (Co-ral). Cattle were poisoned with coumaphos and enzyme systems studied in serum. Some of the cattle received antidotal therapy with 2-PAM. Glutamic dehydrogenase, sorbital dehydrogenase, phosphohexose isomerase and serum arginase were studied in an effort to find significant enzyme activity alterations indicative of possible tissue change. No significant differences were noticed in the enzyme activities regardless of treatment. 2-PAM reduced mortality in coumaphos poisoned animals, but this benefit could not be detected in the enzyme studies. (Kerrville, Texas)

In cooperative work at the Stephen F. Austin State College, Nacogdoches, Texas, the problem of scattered sound intensity from a rigid sphere was programed on the IBM 1620 computer. This showed that calculation errors were previously made in working out this problem. However, the theoretical and observed scattering pattern are still not in agreement.

The relationship of aerosol drop size to the change of sound velocity at various frequencies has been re-done with less experimental error and greatly improved techniques. As yet final calculations on drop sizes determined by this method are not available.

A modification of the Ionovac Speaker has shown that the greatest sound intensity can be obtained from the speaker if it is excited with 23 megacycles RF rather than the design frequency of 27 megacycles. Also the RF excitation does not cause air to ionize but does cause excitation of the nitrogen in the air and thus emission of light.

In other research at this location, solubility studies on potassium antimony tartrate were made at several temperatures. Also the solubility of barium chloride in saturated solutions of potassium antimony tartrate was measured at several temperatures. Apparently two solid phases can exist in this system. Investigation of the composition and properties of each is being continued.

(Nacogdoches, Texas)

(ADP a7-18)

### E. Detoxication Mechanisms in Cattle and Sheep

In studies at the Division's Toxicology Laboratory, Kerrville, Texas, certain oximes have shown an ability to reverse the cholinesterase inhibition induced by organic phosphorus compounds. The ability varies between oximes and between the compounds producing the inhibition.

During this year 2-PAM, DAM, and TMB<sub>4</sub> were the three oximes chosen for study. 2-PAM became available commercially this year. Each of the compounds was useful, but 2-PAM and TMB<sub>4</sub> appeared to be superior to DAM. Particularly encouraging was the beneficial effect of these compounds in cattle poisoned by coumaphos. Usually such animals do not readily respond to atropine, the pharmacologic antidote. Oximes combined with atropine markedly increased the number and speed of recoveries.

In cooperation with the Entomology Research Division, studies of residues of compound 4072 in cattle tissues were made. Spraying cattle with 0.1% emulsions at weekly intervals for 12 applications and at 2-week intervals for 6 applications produced small residues which disappeared within 28 days after the spraying was suspended.

A technique for the analyses of tissue residues of the herbicides 2,4,5-T, propylene glycol butyl ether ester and 2,4,5-T acid has been developed and applied in a test in which three sheep were poisoned by the herbicides. With both compounds residues were deposited primarily as the acid or its salt.

Residues deposited as the acid ranged from an average of 44 ppm in omental fat to 261 ppm in kidney with 73 ppm in muscle and 67 ppm in liver. Some residues were deposited as the ester. The highest was 1.25 ppm in kidney.

The above residues are in animals killed by massive dosing with 2,4,5-T and do not in any way represent the levels which would result from normal exposures. Such studies are planned for fiscal year 1966.

In cooperation with the Entomology Research Division, a number of water samples representing well and surface supplies in the vicinity of the Kerrville Laboratories, were analyzed for chlorinated pesticides. None were found. Sensitivity of the method used would have detected 1 part per billion or less.

(Kerrville, Texas)

(ADP a7-19)

### F. Characterization of Cytological Response to Toxic Actions of Anti-parasitic and Other Agricultural Chemicals in Cattle and Sheep

At the Division's Toxicology Laboratory, Kerrville, Texas, research studies indicated that the polyfunctional alkylating agents apholate, tepa, and metepa, which are insect chemosterilants, injected into the yolk of fertile eggs just prior to incubation and into the yolk sacs of developing embryos after varying periods of incubation, induced congenital abnormalities in embryos that survived to the 18th day of incubation.



Doses of each compound at 250 µg. and higher per egg were lethal to 4-day-old embryos. Death usually occurred within 72 hours. Each compound at 125 µg. per egg was lethal to 1- and 2-day-old embryos in 72 - 96 hours, but allowed 4-day-old embryos to continue to develop for as long as 11 days before they died. Doses of each compound at 5.4 to 25 µg. per egg usually permitted embryos to develop. Each compound induced similar congenital abnormalities such as defects of the beak, eyes, digits, and legs; cerebral and visceral hernia; edema; growth retardation and reduced weight.

(Kerrville, Texas) (ADP a7-20)

#### G. Livestock Poisoning by Plants

Researchers at the Division's Poisonous Plants Laboratory at Logan, Utah, report on their work as follows:

Cyclopian-Type Malformation in Lambs. The teratogenic material of the plant Veratrum californicum, responsible for ovine fetal cyclopsia, has been subjected to extensive purification. Over 40 malformations and fetal deaths were produced with extracts and purified materials. A number of glycosides and parent alkaline steroidal alkaloids were isolated by fractional crystallization. Preliminary studies indicated that two of the glycosides and two of the parent alkalines were capable of producing the teratogenic effect. Infrared studies have allowed a limited correlation of the structural requirements for teratogenicity. (ADP a7-7)

(Logan, Utah and Ames, Iowa)

Physiopathologic Aspects of Lupinus sericeus and L. caudatus (crooked calf syndrome). The crooked calf syndrome continues to be a wide-spread problem throughout the Western States and Alaska. This disease entity has not been characterized and is often confused with other deformities such as bovine achondroplasia, arthrogryposis, internal and external hydrocephalus, prognathia, anophthalmia, cerebellar ataxia, contracture of tendons and spastic lethals.

The degree of skeletal-musculo deformities varies greatly in individual cases. If the malalignment and malpositioning of the limbs and vertebrae is not too severe, they appear to become apparently normal clinically as the animal matures. If the deformity is too severe at birth, then the malformation is exaggerated and becomes progressively worse as the animal matures.

This disease syndrome is very complex and evaluation of the lesions can be justly open to more than one interpretation. The musculo-skeletal system is structurally and functionally a unit, it can be fully understood only if this concept is kept in mind while artificially separated parts of it are studied.



Feeding of Lupine plant to pregnant Hereford heifers from the 22nd to 120th days of gestation caused congenital deformities and abortion. The malformed calves were characteristic of clinical field cases of the crooked calf syndrome.

The crooked calf syndrome has a wide spectrum of lesions. There appears to be some inhibition, arrest, or interference with the normal sequential differentiation and specialization of cells, tissues, and organs at a specific time in embryonic and fetal development. The spectrum of lesions indicates the time of insult may occur at different stages of embryogenesis. Limb malalignment and vertebral aplasia are characteristic of this disease entity. Anophthalmia and cyclopia have been seen in calves with skeletal lesions typical of this syndrome. Flexure of pastern joints can be differentiated from this condition by clinical history, signs and x-ray evaluations.

Definite management practices can be correlated with the crooked calf syndrome. Cows grazing irrigated and crested-wheat grass pastures all had normal calves, while cows grazing in Newman Canyon had a number of crooked-legged calves. Lupine taken from this range area was used to experimentally produce a congenital malformed calf typical of clinical field cases. There was no correlation between bulls used and the incidence of deformed calves. All abnormal calves were born during a definite short period of time in the early part of the normal calving season.

Numerous uncontrollable factors are present on range areas where the crooked calf syndrome is a problem. Seasonal variation of such factors could influence the seasonal incidence of the disease.

Based on information and data accumulated to date, the crooked calf syndrome appears to be non-hereditary in nature, however, associated with the ingestion of some toxic substance and/or substances during some stage of embryonic or fetal development.

If the cause and/or causes and lesions associated with the crooked calf syndrome can be more fully understood, it will be invaluable in a number of ways. It will save livestockmen large sums of money and permit wiser utilization of range areas. (Logan, Utah) (ADP al-28)

#### H. Studies to Develop Alleviators and Diagnostic Tests for Plant Poisoning, and Methods to Avoid Harmful Residues in Animal Tissues from Ingesting Chemically Treated Plants.

Researchers at the Division's Poisonous Plants Laboratory, Logan, Utah, report that feeding False Hellebore (Veratrum californicum) to ewes 7 and 21 days after being sprayed with 2-4D Ester and 2-4D Amine reduced the incidence but did not completely eliminate the plant's ability to cause congenital deformities when ingested by ewes on the 14th day of gestation.

The total amount of alkaloids in the veratrum plants, determined on dry weight basis, was not significantly decreased from the herbicidal treatments.

Further investigation will be made with other herbicides in cooperation with the Crops Research Division. (Logan, Utah) (ADP a7-17)

#### I. Mycotic Diseases of Domestic Animals

At the National Animal Disease Laboratory, Ames, Iowa, researchers on this subject report as follows:

Nocardiosis is an important infectious disease of cattle, dogs, and man. Systemic nocardiosis frequently mimics tuberculosis or systemic fungal infection and diagnosis is often delayed until the disease has reached terminal stages. An antigen has been developed and used successfully in skin tests, complement fixation tests, and agar gel precipitin tests of cattle experimentally infected with Nocardia asteroides. Methods for the standardized preparation and fractionation of the culture filtrate antigen were developed and the antigenic principal was found to reside in a single column fraction. This fraction was found to be active in immunologic tests for delayed cutaneous hypersensitivity, complement-fixing and precipitating humoral antibodies. Chemical analysis of the antigenic material indicated that its major constituents were protein (55%) and hexose (10%). Neither chemical nor immunologic evidence of cell wall constituents were detected - the latter evidence is of significance to the antigen's specificity. Further work to determine the range of specificity of this antigen is contemplated. (Ames, Iowa) (ADP a7-24)

#### J. Biological Changes Associated with Neuropathological Conditions in Animals

At the National Animal Disease Laboratory, Ames, Iowa, the mechanism of the production of hyperglycemia by Veratrum alkaloids has been studied in adrenalectomized sheep and in sheep treated with chemical adrenal blocking agents. Neither dihydroergotamine nor N-isopropyl methoxamine prevented hyperglycemia or other clinical effects of the alkaloid. In vitro studies with sheep tissue slices suggested that glucose metabolism was inhibited when tissues were incubated with the alkaloids. (Ames, Iowa) (ADP a7-26)

#### K. Physiological Investigations of the Interrelation between the Respiratory, Circulatory and Digestive Systems of Animals

At the National Animal Disease Laboratory, Ames, Iowa, work with the intravenous or intramuscular injections of ester alkaloid preparations from the plants Veratrum viride and V. californicum (0.2 to 10.0 mg/100 kg) resulted in vomition. Somewhat lower doses were effective in relief of non-frothy bloat in cattle within 5 to 10 minutes. However, the preparations were less effective in relief of frothy bloat. Doses effective in relieving bloat, enhancing rumination and stimulating vomition produced only minimal temporary muscular incoordination and hypotension which ceased within one hour.



Toxic factors chemically and physiologically similar to bacterial lipopolysaccharide endotoxin have been extracted from rumen bacteria and from cell-free rumen liquor. The similarities in physiologic response of sheep to intravenous administration of these materials with that of animals suffering from grain engorgement suggest that endotoxins from normal rumen microorganisms may play a significant role in the disease.

(Ames, Iowa) (ADP a7-27)

L. Proteins and Other Complex Molecules from Animal Disease Agents Derived Primarily from Surface Structures and Extracellular Products

Researchers at the National Animal Disease Laboratory, Ames, Iowa, found isolated flagella from two different biotype strains of Vibrio fetus to be antigenically identical and identical in chemical composition. Peptide maps, amino acid composition, N-terminal amino acid, and electrophoretic criteria were used to establish chemical compositional identity, while Ouchterlony gel diffusion was used to establish antigenic identity.

Two common soluble intracellular antigens from three bio-serotypically different Vibrio fetus strains have been extensively purified and are being subjected to chemical comparison. (Ames, Iowa) (ADP a7-28)

M. Chemical and Physical Studies on Microbial Antigens

At the National Animal Disease Laboratory, Ames, Iowa, studies have been completed on the characterization of several antigens. Formalinized saline extracts of cells of two strains of Pasteurella multocida of avian origin were fractionated by ultracentrifugation. A gel-like precipitate which had both toxic and immunizing properties for chickens and mice was obtained. A marked increase in the specificity of the serological reactions was obtained with the gel-like precipitate as compared to the unfractionated saline extract.

A nitrogen and carbohydrate-containing antigen has been isolated from the culture supernatant of Actinomyces bovis and purified by alcohol fractionation and column chromatography. (Ames, Iowa) (ADP a7-29)

N. Microbiology of the Ruminant Digestive Tract and its Relation to Digestive Disturbances

At the National Animal Disease Laboratory, Ames, Iowa, a new pathway for biosynthesis of phenylalanine has been demonstrated. Many pure cultures of rumen bacteria, the mixed ruminal population incubated in vitro, and also certain nonruminal anaerobic bacteria synthesize phenylalanine using the intact carbon skeleton of phenylacetic acid. Details of the biosynthetic mechanism are not known, but there is evidence suggesting a condensation of the carboxyl-carbon of phenylacetic acid with  $\text{CO}_2$  to produce phenylpyruvic acid, which is then transaminated. (This is different from the previously described biosynthetic pathway shown in aerobic microorganisms



where the 3-carbon side chain of phenylalanine arises as a unit from an intermediate in glycolysis.) Limited data indicate that, in the rumen, tryptophan is synthesized from indoleacetic acid, presumably also by a carboxyl-carbon-CO<sub>2</sub> condensation reaction. (This is different from any known pathway of tryptophan biosynthesis.) These are apparently significant reactions and reflect microbial adaptation to the ruminal environment where phenylacetic and indoleacetic acid are usually present in appreciable quantities, while free phenylalanine and tryptophan are not.

Toxic factors chemically and physiologically similar to bacterial lipopolysaccharide endotoxin have been extracted from rumen bacteria and from cell-free rumen liquor. The similarities in physiologic response of sheep to intravenous administration of these materials with the physiology of animals suffering from grain engorgement suggest that endotoxins from normal rumen microorganisms may play a significant role in the disease. Intraruminal administration of endotoxin had little effect on sheep and conditions permitting absorption of intact endotoxin from the gastro-intestinal tract are not known.

(Ames, Iowa) (ADP a7-30)

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TREATMENT FOR REMOVAL OF PARASITES  
OF DOMESTIC ANIMALS  
Animal Disease and Parasite Research Division, ARS

Problem. Parasites of food animals are responsible for losses to livestock producers approximating a billion dollars annually. This estimate, moreover, is conservative since it does not take into account costs of treatment and other control measures. Chemical antiparasitic agents are the most powerful weapons presently available against parasites and the diseases they cause, yet specific treatments generally have a comparatively short period of usefulness. Many of the currently preferred treatments were unknown a decade or so ago and, in all probability few, if any of those in use today will be primary choices a decade or so hence. Moreover, the growing concern with respect to residues in edible tissues and organs of treated animals and birds necessitates development of control measures other than treatment. The problem is to develop, through a planned, balanced program of basic and applied research control methods that minimize reliance on extrinsic chemicals. These include investigations of immunological procedures, management practices which minimize exposure of animals to parasitic infections, and natural control agents such as parasites, pathogenic microorganisms, and predators of economically important livestock pests.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving veterinarians, parasitologists, pharmacologists, and biochemists engaged in both basic studies and the application of known principles in developing treatments for removal or control of parasites of domestic animals. Research is being conducted on this problem at the following designated locations.

The Federal scientific effort devoted to research in this area totals 9.5 professional man-years. This effort is applied as follows:

Chemical Control of Parasitic Diseases 1.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

New and Improved Anthelmintics 3.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Hazards of Residues from Treatment for Parasites 3.5 at the Regional Animal Disease Research Laboratory, Auburn, Alabama.

Pathobiology of Parasitic Infections 1.0 at the Albuquerque, New Mexico, field station.

Control and Eradication of Scabies 0.5 at the Albuquerque, New Mexico, field station.

## PROGRAM OF STATE EXPERIMENT STATIONS

State research in this area is designed to provide detailed information needed for the safe and effective application of chemicals in the control of livestock parasites. Promising new compounds are evaluated singly and in various combinations to determine effective treatment against the important species of livestock parasites. Major emphasis is being placed on treatment of infections due to nematodes. Other work is directed toward therapeutic control of coccidia, histomonads, liver flukes and tapeworms.

Methods of administering anthelmintic compounds and dosages required for the most effective parasite control are evaluated along with considerations of toxic effects which the drugs may have on host animals. Potentially useful compounds are being compared for effectiveness as measured by reductions in parasites and parasite eggs and increases in weight gains and feed efficiency in the treated host. Simplified methods for administering anthelmintics on a herd or flock basis are being developed and the use of these compounds is being coordinated with research on effective management methods.

Basic research at several locations is seeking fundamental information on how anthelmintics act upon biochemical systems involved in parasite metabolism. The problem of drug resistance in certain strains of parasites also is under study.

The States are allocating 15.2 professional man-years to research on the treatment of livestock parasites.

## PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Investigations of dimetridazole and other potential chemotherapeutic agents as treatments for bovine venereal trichomoniasis.

In research work at the Beltsville Parasitological Laboratory, Trichomonas foetus infections were eliminated from 6 bulls by the oral administration of dimetridazole at the rate of 50 mg/kg of body weight daily for 5 consecutive days. The chemical was given either by capsule or admixed with the feed. Five daily doses at 25 mg/kg administered by capsule freed another bull of T. foetus, but this level was ineffective when fed in grain to 2 bulls. One of the latter subsequently became negative after repeated courses of treatment at higher dosages. The other bull retained the infection.

Research efforts with dimetridazole during the current year have been concentrated on the development of a safe and reliable treatment involving a single administration of the chemical. Trichomonads have not been recovered on repeated examinations of 5 bulls that were each given only one intravenous injection. Two other bulls required a second treatment at a higher dosage level before they became negative for T. foetus. The chemical is well tolerated at all dosage levels. (BPL) (ADP b5-19)



B. Evaluation, development, and standardization of chemical methods of established or reported value for the control of parasitic diseases of livestock and poultry.

Researchers at the Beltsville Parasitological Laboratory report that nihydrazone, at a level of 0.011% in the feed of chickens with experimental Eimeria tenella infections, greatly reduced mortality and oocyst production. However, the incidence of cecal lesions was high and the growth of infected birds was poor. A similar level of medication did not inhibit the growth of uninfected birds.

Strains of Eimeria tenella, passed through 58 successive groups of chickens medicated with Unistat or arsenosobenzene, respectively, developed a pronounced tolerance to the specific drugs. The degree of tolerance was greater in the Unistat-medicated strain than in the one exposed to arsenosobenzene.

A strain of E. tenella serially exposed to amprolium for 48 generations developed a distinct tolerance to the chemical. Beginning with the 39th passage, there were occasional deaths among the infected, medicated birds.

Unsporulated oocysts of E. tenella were not adversely affected by storage at refrigerator temperatures for 3 months. The rate of sporulation and virulence were comparable to those of conventional cultures.

Dimetridazole administered in the drinking water for 3 days, eliminated Trichomonas gallinae infections, the cause of canker in pigeons.

Piperazines are excellent drugs for removal of the large roundworm, Ascaridia galli, from chickens, but they proved to be only moderately effective against Ascaridia columbae of pigeons. Dose rates equal to or higher than those that are ordinarily very effective against the chicken ascarid failed to achieve comparable efficacy against the large roundworm in pigeons. The drugs stupify the worms and their removal is effected by intestinal peristalsis. Accordingly, if peristalsis is impeded for any reason, the effects of the drugs may disappear before the worms can be expelled. Because the intestine of the chicken is much larger than that of the pigeon, interference with peristalsis may be less likely to occur. This may account, at least in part, for the difference in efficiency of piperazines in the two hosts.

Limited tests with subcutaneous injections of methyridine, a new systemic anthelmintic, indicate that this chemical may compare favorably with other trichuricides for dogs. A dosage of 150 mg/kg of body weight was completely effective against 348 Trichuris in 4 dogs, and at 200 mg/kg the drug removed all of 385 Trichuris from 6 dogs. The larger dosage was presumably fully effective also in 5 additional dogs that were not necropsied.

Moderate activity was exhibited against Ancylostoma in limited trials at the 200-mg level. Anthelmintic action against Toxocara, Taenia, and



Dipylidium was either negligible or too limited to permit even provisional interpretations of efficacy.

Emesis, ataxia, soft feces, and acute irritation at the site of injection were evidenced at all dosage levels. These reactions, however, were transitory.

(BPL)

(ADP b5-5)

Field trials in Mississippi, conducted by researchers at the Regional Animal Disease Laboratory, Auburn, Alabama, indicated that Thibenzole was an effective anthelmintic in lambs when administered monthly in the feed. At a dose rate of 44 mg/kg of body weight, Thibenzole controlled nematode parasites of lambs as well as drenchings with phenothiazine with the exception of Haemonchus contortus. The ease of administration of Thibenzole in this manner, eliminating the need for individual treatment, would favor its use, especially in areas where H. contortus is not of primary concern.

(Auburn, Alabama)

(ADP b5-5(Rev.))

C. Investigations to develop new and improved chemical agents for the treatment, prevention, or control of helminthic parasites in farm animals.

At the Beltsville Parasitological Laboratory, researchers found that an 0.5% thiabendazole-medicated mash was remarkably effective in removing the large intestinal roundworm, Ascaridia columbae, from experimentally infected pigeons. Medicated mash was fed ad libitum for approximately 1 to 2 weeks, and the treatment removed, in the aggregate, 95% (1,522) of 1,592 worms from 57 birds. The drug exhibited anthelmintic action against both immature and mature worms. This is the first satisfactory treatment developed for the removal of Ascaridia columbae from pigeons.

Limited tests with subcutaneous injections of methyridine, a new systemic anthelmintic, indicate that this chemical may compare favorably with other trichuricides for dogs. A dosage of 150 mg/kg of body weight was completely effective against 348 Trichuris in 4 dogs, and at 200 mg/kg, the drug removed all of 385 Trichuris from 6 dogs. The larger dosage was presumably fully effective also in 5 additional dogs that were not necropsied.

Moderate activity was exhibited against Ancylostoma in limited trials at the 200-mg level. Anthelmintic action against Toxocara, Taenia, and Dipylidium was either negligible or too limited to permit even provisional interpretations of efficacy.

Emesis, ataxia, soft feces, and acute irritation at the site of injection were evidenced at all dosage levels. These reactions, however, were transitory.

In limited trials, methyridine, a systemic anthelmintic, exhibited marked action against the intestinal capillariid, Capillaria obsignata, in pigeons. Doses of 45 mg, administered subcutaneously, removed all of 1,390 Capillaria from 10 birds. Doses of 35 mg were about equally effective, removing 99%

of 1,667 capillariids from 6 birds. A slight swelling at the site of injection and a temporary incoordination were the only untoward signs observed.

Capillaria obsignata has been responsible for outbreaks of capillariasis among chickens, particularly laying hens, in the United States as well as in other countries. The parasite also occurs in turkeys and peafowl.

(BPL)

(ADP b5-18)

D. Control of Internal Parasites of Livestock by Management Practices that will not Create Consumer Residue Hazards.

At the Experiment, Georgia, substation of the Regional Research Laboratory, Auburn, Alabama, a study was continued on the epidemiology of helminthiasis in sheep in Georgia. So far, September appears to be the most critical month for grazing pastures, as evidenced by the larger number of eggs passed during that month. Judging from egg counts, younger animals were more heavily parasitized than older ones. (Auburn, Alabama) (ADP b5-16)

E. Pathobiology of Parasitic Infections with Special Reference to the Injuriousness of Arthropod Parasites, and the Economic Gain and Efficiency of Control Measures.

At the Regional Research Laboratory, Albuquerque, New Mexico, work was directed toward the screening of candidate parasitocides and investigations into methods of administration for the control of lice on cattle. Livestock pesticides are most efficient, and are most widely accepted by stockmen, when they are easily applied and destroy several parasites rather than merely one. In a search for compounds and methods of administration applicable to the simultaneous control of cattle grubs, horn flies, and lice, several materials were evaluated from the standpoint of their lousicidal effectiveness on cattle. Famphur, administered orally in the feed, daily, proved 100% effective in destroying sucking lice on cattle, but did not eliminate biting lice, and was completely ineffective against scab mites. Collars and belts impregnated with Vapona proved 100% effective in eliminating both sucking and biting lice from cattle. Compound SD 8447, applied as a pour-on to the backs of cattle, was not effective in controlling lice. Further work with Famphur and Vapona against lice on cattle is strongly indicated.

(Albuquerque, New Mexico) (ADP b5-13)

F. Development of New Approaches and Methods for the Control and Eradication of Scabies in Sheep and Cattle.

At the Regional Research Laboratory, Albuquerque, New Mexico, continued observations were made on the comparative pathogenicity of various strains of Psoroptes ovis. Completed aspects of studies on sheep, involving various field strains of Psoroptes ovis, the mite responsible for common scabies of sheep, cattle, and horses, show conclusively that differences in virulence or aggressiveness among strains are profound. Such virulent



strains are not only highly pathogenic to sheep, but show evidence of resistance to drugs as well. The practical contribution of these investigations has been to provide material for the perpetuation of a dependable source of infested hosts in various stages of severe clinical parasitism, suitable for the screening and evaluation of candidate and established acaricides. Furthermore, maintenance of discrete strains broadened old concepts regarding clinical manifestations of psoroptic acariasis, while inbreeding and outcrossing of strains may lead to advances in the field of arthropod parasite strain exhaustion and host-resistance.

Psorergatic acariasis was discovered on a Hereford cow in New Mexico in 1963. Subsequently, 14 other cases from 7 different premises in New Mexico and Texas were found. Attempts to transfer Psorergates bos from the original infested cow to other cattle, and to white rats and rabbits, were unsuccessful. Gross clinical manifestations of acariasis have not been associated with the presence of the mites. There is reason to believe Psorergates bos to be a widely distributed parasite of cattle which, due to its small size and apparent inoffensiveness to its host, has heretofore escaped detection.

Psorergates ovis, the Australian itch mite, was found on a New Mexico range ewe. The infestation was characterized by alopecia and broken, matted fleece. Mites in all stages of development were found in numerous skin scrapings but could not be found by histologic examination of skin sections. Efforts to transmit the mites from the ewe to rabbits and white rats were unsuccessful. The infestation was, however, successfully transmitted to 2 unshorn lambs in close confinement with the unshorn subject for 6 to 8 months. After all sheep involved were shorn, transmission to a ewe required less than 4 months.

Subsequently, the natural transference of the parasite to 4 additional sheep was accomplished, but efforts to infest goats and calves, through contact, failed. Since the mite does transfer from sheep to sheep without inordinate difficulty, because it was found in a Southwestern range flock not otherwise remarkable, and since its discovery was the third reported in the United States, it is possible that its distribution may be more widespread than is currently suspected.

In work on the blood cell responses in the sheep to Psoroptic scabies, studies on the blood of non-scabby sheep, which are not otherwise heavily parasitized, such as range sheep commonly encountered in the Southwest, indicate that lymphocytes and neutrophils, the most numerous white cells, appear in almost equal numbers, while monocytes outnumber eosinophiles as much as 10:1. However, when these sheep are exposed to common scab, the eosinophiles outnumber the monocytes even before clinical symptoms of scab are detectable. As the disease progresses, the eosinophiles come to outnumber the monocytes as much as 30:1 or more. No predictable change in the relative numbers of lymphocytes and neutrophils were observed.



In observations on the host-parasite relationship of Psoroptes ovis to sheep, it was found that when sheep infested with scab mites are isolated from other flock members, the parasites appear invariably to die, and the sheep spontaneously recover from scabies. Certain phases of the study of this phenomenon, completed in fiscal year 1965, suggest that the virulence or disease-producing capacity of the mites involved, and the status of clinical infestation at the time of isolation, have no bearing on the duration of survival of mite populations. The reason for mite population extinction is as yet unknown, but now appears to be more closely associated with host response than with parasite strain exhaustion.

(Albuquerque, New Mexico) (ADP b5-15)

#### PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

##### Bovine Veneral Trichomoniasis

McLoughlin, D. K. 1964. Activity of dimetridazole in Trichomonas foetus infections. J. Parasit., 50:2:57.

##### Control of parasitic diseases of livestock and poultry

Baird, D. M., White, P. E., Ciordia, H., Bizzell, W. E., and McCampbell, H.C. 1964. Low-level medicated mineral for the control of horn flies and cattle grubs. Georgia Agr. Exp. Stations, Mimeo series N.S. 205, 9pp.

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MISCELLANEOUS PARASITES AND PARASITIC DISEASES  
Animal Disease and Parasite Research Division, ARS

Problem. Parasitism is a way of life that characterizes the majority of living things. Except for basic life processes, it is probably the commonest biological phenomenon. More than 50,000 kinds of animal parasites (i.e., parasites classified as animals as opposed to those classified as plants) are known. New varieties are being discovered and described at a rate of about 500 per year. Some devastating parasites, indigenous to foreign countries, threaten to surmount barriers imposed against them. Certain of these have already gained new footholds in livestock, poultry, and wildlife. Essential elements of procedure against parasites--established, exotic, or new--are accurate diagnosis, development of full knowledge about them, and research on effective control measures. The primary requirement is development through research of up-to-date knowledge of classification and identification supported by a complete reference collection of parasites, including type specimens and familiarity with global research already done. Basic investigations of parasitisms as biological phenomena are involved, especially in host-parasite relations, immunology, serology, ultrastructure, and other aspects of diagnosis and control. The problem is to develop and maintain up-to-date methods of identification and the essential, supporting reference collections, as well as complete parasitological information extracted from the world's scientific literature; investigate important phenomena and host-parasite systems not covered in specific host categories; and provide bases for detection and control that are adequate to meet existing and anticipated needs, through research on problems involving various parasites and hosts, including wild animals and birds important to agriculture.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program for parasitologists, biochemists, and microbiologists, engaged in basic and applied research in this area. Research is being conducted on the following problems at the designated locations.

The Federal scientific effort devoted to research in this area totals 10.5 professional man-years. This effort is divided among subheadings as follows:

Maintenance and publication of author, subject, and host index-catalogues 2.5 at the Beltsville Parasitological Laboratory.

Immunologic and other biologic approaches to the prevention and control of parasitic diseases 3.0 at the Beltsville Parasitological Laboratory.

Chemical and physical elements of parasites and parasite-host relationship 2.0 at the Beltsville Parasitological Laboratory.

Taxonomic investigations of parasites 2.0 at the Beltsville Parasitological Laboratory.

Maintenance of parasite collection 1.0 at the Beltsville Parasitological Laboratory.

#### PROGRAM OF STATE EXPERIMENT STATIONS

Information is being compiled at a number of States on the incidence and importance of specific parasites of livestock. Several States have work dealing with the morphology and comparative anatomy of parasites to aid in identification and classification of the species involved. Research is in progress at several locations on the laboratory culture of adult parasitic nematodes. Basic information is being obtained on the nutritional requirements of these parasites and the products of metabolism are being evaluated as potential immunizing agents.

The States have 7.5 professional man-years allocated to this area.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Maintenance and Publication of Author, Parasite-Subject, Host, and Anthelmintic Catalogues.

At the Division's Beltsville Parasitological Laboratory, the Index-Catalogue of Medical and Veterinary Zoology has been maintained and expanded in its various sections - Author, Parasite-Subject, and Host Catalogues, and Check-list of Specific and Subspecific Names. New entries augmenting the Catalogues are as follows: Author entries, 8,672; Parasite-subject entries, 24,873 (including 22,560 Parasite entries, 2,313 Anthelmintic entries); and Host entries, 6,303. The Index-Catalogue has continued to supply references for the Anthelmintic Catalogue of the Anti-parasitic Investigations. Literature on plant parasitic nematodes was fully indexed for about the first half of the year - since that time only taxonomic papers on this group have been indexed.

New genera and species of parasites are as follows: Protozoa: 7 n.g., 149 n. sp.; Trematoda: 36 n.g., 145 n. sp.; Cestoda: 6 n.g., 47 n. sp.; Nematoda: 85 n.g., 607 n. sp.; Arthropoda and miscellaneous groups: 23 n. g., 373 n. sp. There have been 204 new citations of periodicals added to the Catalogue. An average of 500 periodicals are examined each day at the National Agricultural Library for parasitological papers to be indexed for the Index-Catalogue.

The Index-Catalogue has had more than 40 visitors from the United States and eight other countries, some of them staying several days and consulting it as a source of information. (ADP b6-14)



Supplement 14, Authors: A to Z, was published in September, 1964, and Supplement 15, Authors: A to Z, was published in March, 1965. Material for the Protozoa and Trematoda parts of the Parasite-Subject Section of Supplement 15 is being processed for photo-offset printing. Part 3: Supergenera and Genera D of Subjects: Trematoda and Trematode Diseases, was published in the fall of 1964. (BPL) (ADP b6-9)

B. Immunologic and other biologic approaches to the prevention and control of parasitic diseases.

At the Beltsville Parasitological Laboratory, attempts to separate and concentrate antibodies to Stephanurus dentatus by gradual thawing of frozen serum during centrifugation were partly successful. Antibodies to excretory gland extract were concentrated 4-fold in fractions containing the heavier serum protein components. Antibodies to intestinal extract were present in approximately equal concentrations in all fractions.

Studies were continued on the in vitro growth of Stephanurus dentatus in an attempt to extend development beyond the fourth stage and to evaluate various ingredients. The findings were evaluated by comparing the results with data previously obtained using medium PB-1 enriched with NCTC-109. The data showed that vitamin B-12 was essential to the development of S. dentatus. In the absence of this factor, living deformed larvae occurred in the third and fourth stages. Although it was shown previously that serum was essential for development to the fourth stage, new data showed that a serum concentration of 50% was more beneficial than one of only 12.5 percent. Greater yields of fourth stage S. dentatus were obtained in KW-1, a modification of PB-109 formulated by omitting the balanced salt solution and increasing the serum to 50 percent. Development of S. dentatus was not enhanced by the addition of sterilized, heat-dried, meat-egg medium, meat-egg-phytone, and liver extracts, and suspensions of fresh, trypsinized swine kidney and liver cells to cultures of the parasite developing in KW-1. However, evidence was obtained that fourth stage larvae actively feed on particles of meat-egg and on kidney and liver cells.

Studies were continued on the in vitro growth of Oesophagostomum radiatum in an attempt to extend development beyond the fourth molt and to evaluate various ingredients. Experiments designed to study the effect of environmental conditions showed that O. radiatum larvae developed in 59 days to young adults, a stage not previously attained, when grown in screw-capped Erlenmeyer flasks containing 20 ml of medium SM-1 and incubated in an upright, stationary position at 38.5 C. In earlier studies, it was shown that the larvae developed only to fourth molt when cultured in screw-capped tubes containing 3 ml of SM-1 and incubated in a horizontal, stationary position. Using the same protocols, the effects of free gas exchange (cotton-plugged flasks) and anaerobiasis (5% CO<sub>2</sub> -95% N<sub>2</sub>) were studied. Both of these environmental conditions inhibited development to the fourth stage and caused a death rate of 95% of the total inoculum by the 34th day in the cotton-plugged flasks and by the 38th day in the anaerobic flasks.

Development comparable to that obtained in cultures maintained in screw-capped flasks was attained when cotton-plugged and anaerobic cultures were changed to screw-capped vessels. Evidence also showed that after several transfers under anaerobic conditions, cultures removed to screw-capped flasks underwent synchronous development to each advanced stage.

In the nutritive trials, the addition of autoclaved suspensions of hemoglobin, egg-meat medium, and gastric mucosa to medium SM-1 containing larval cultures in all stages up to fourth molt resulted in the death of larvae in fourth stage and fourth molt and inhibited development of larvae in parasitic third stage and third molt.

Studies on the in vitro growth of Haemonchus contortus in medium SM-1 were continued in an attempt to extend development beyond the fourth molt by changing the environmental cultivation protocols. The findings were evaluated by comparing the results with data previously obtained by the tube-method. Using screw-capped flasks, H. contortus advanced to the fifth stage or young adults in 19 days. Although survival could not be extended beyond the 40th day, advancement to fifth stage represents significant progress since this stage had not been previously attained.

A first attempt has been made to grow Oesophagostomum columbianum through its parasitic stages. Cultured with H. contortus in screw-capped flasks containing SM-1, O. columbianum developed through early to late parasitic third stage, third molt, early and late fourth stages and fourth molt as early as 4, 11, 11, 18, and 35 days in culture, respectively. After 35 days, larvae in all stages appeared starved.

Oral vaccination with infective larvae of Dictyocaulus filaria of sheep was tested as a means for the protection of calves against infection with the cattle lungworm, D. viviparus. In three small-scale exploratory trials, five calves were vaccinated and five served as controls. Dosages of D. filaria larvae, numbers of vaccinations, intervals between final vaccination and challenge, and levels of challenge, differed between trials. Each of the controls acquired a substantial patent D. viviparus infection from a challenge dose of larvae, whereas four of the five comparably exposed vaccinated individuals were highly resistant or immune to the establishment of mature D. viviparus. D. filaria apparently did not mature in the infected calves. The smallest dosage per vaccination given to young calves was 5,000 larvae. It was too pathogenic for use for immunization in early calfhood. Short yearlings tolerated repeated vaccination with about 2,000 larvae very well.

(Beltsville, Maryland) ADP b6-10)

#### C. Chemical and Physical Elements of Parasites and Parasite-host Relationship.

At the Beltsville Parasitological Laboratory, extracts from the excretory glands of swine kidney worms and serums from kidney-worm-infected and control swine were fractionated by density-gradient ultracentrifugation. When the



extracts are layered on a sucrose solution which increases in concentration from top to bottom and the whole is subjected to prolonged (18 hours) centrifuging at ultra speeds, the antigenic components of the extract are distributed from near the top to about three-fourths of the way down the column of fluid. The reactive antibodies of serum under the same treatment are distributed about half way down the column. The antibodies from control swine react with antigens which sediment only slightly, while the antibodies from the infected swine react with antigens over the whole range.

Considerable volumes (3 - 15 mls.) of solutions of kidney worm antigens of improved specificity were prepared. This was done by collecting and concentrating those antigenic components of a mixture which migrate toward the positive electrode of the "barrier electrophoresis" apparatus.

Comparison of the red pigment of gapeworm with hemoglobin from the host birds, turkeys, reveals that the two pigments differ in their sedimentation properties in the ultracentrifuge, their migration rates in an electric field, and in their susceptibility to denaturation by strongly alkaline solutions or by the drug barium antimonyl tartrate. The worm pigment contains the same percentage of iron as does hemoglobin.

(Beltsville, Maryland) (ADP b6-11)

#### D. Taxonomic investigations of Helminths and other parasites.

Three manuscripts were prepared for publication. A new species of hookworm was described from specimens recovered from the small intestine of deer in Louisiana and the description is supplemented with a key to the seven species in the hookworm genus Monodontus. A new genus and a new species of a spiruroid nematode were described from specimens recovered from the stomach of peccaries in Texas and New Mexico, and a new species of Nematodirus was described from specimens collected from the small intestine of mountain goats in Montana and Alberta.

(BPL)

(ADP b6-12)

At the Beltsville Parasitological Laboratory, 317 lots of specimens were identified (protozoans 1, trematodes 12, cestodes 7, acanthocephalans 3, nematodes 173, and arthropods 121). Among these were numerous parasites of medical and veterinary importance. One lot of nematodes recovered from the intestine of a human patient in the Philippines represents a new species and the first case of intestinal parasitism by a species of Capillaria in man. A mature male meningeal worm, Odocoileostrongylus tenuis, a nematode previously reported in the brain and spinal cord of sheep, deer, and moose in North America, was found in a pituitary gland removed from a deer in Georgia. Th

The following parasites were collected from animals and items offered for entry into the United States: (Ticks) Amblyomma cajennense, A. dissimile, A. gemma, D. variegatum, Boophilus decoloratus, B. microplus, Dermacentor sp. probably D. everstianus, D. nigrolineatus, D. nitens, D. parumapertus, Hyalomma anatolicum, H. marginatum, Ixodes hexagonus, I. scapularis,



Ornithodoros megnini, Rhipicephalus bursa, R. evertsi, and R. pulchellus;  
(Lice) Haematopinus eurytarnus and Linognathus africanus; (Flies) Hippo-  
bosca sp. and Melophagus ovinus. (Beltsville, Maryland) (ADP b6-16)

#### E. Maintenance of Parasite Collection.

At the Beltsville Parasitological Laboratory, 731 lots of specimens  
(protozoans 1, trematodes 164, cestodes 50, acanthocephalans 12, nematodes  
339, arthropods 139, and miscellaneous 16) were added to the parasite  
collection. (BPL) (ADP b6-15)

### PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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and Veterinary Zoology, Supplement 14, Authors: A-Z. U. S. Government  
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#### Immunologic Approaches to Parasitic Diseases.

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Rose, J. E., Baisden, L. A., and Tromba, F. G. 1964. Ultracentrifugal  
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**BEEF CATTLE, HORSE, AND SWINE INSECTS**  
**Entomology Research Division, ARS**

Problem. Insects and ticks irritate and torment livestock throughout the year in all parts of the United States and cause serious losses. These pests reduce weight gains, lower the quality of meat and hides, and spread numerous animal diseases. Livestock losses directly attributable to insects and ticks are estimated to exceed \$300 million annually. Practical but not adequate control methods have been developed for lice, screw-worms, ticks, bots, grubs, and other insects, but satisfactory methods of protecting cattle from horse flies, deer flies, stable flies, mosquitoes, and the newly introduced face fly remain an unsolved problem. The development of insecticides for use on beef cattle, horses, and swine has been hampered because certain insects have become resistant to various insecticides, and because harmful residues have been found in meat following the application of certain materials. Safe, effective, nonresidue-forming insecticides and repellents are required. Urgently needed are economical and long-lasting insecticides or repellents for range cattle to protect them against vicious biting flies. Safer, cheaper, and more effective systemic insecticides and more efficient means of administration are needed to combat grubs and bots in cattle and horses. New approaches to control, including radiation and chemosterilants, should be explored to determine their feasibility as practical control methods. Efforts also need to be made to find and evaluate insect pathogens, parasites, and predators for controlling certain livestock pests. Expanded basic studies on the biology and physiology of these pests are needed to find weak links in their life cycles that will serve as a basis for the development of more effective and safer methods of control. Research also is urgently needed on the role of insects in the spread of diseases of livestock.

**USDA AND COOPERATIVE PROGRAM**

The Department has a continuing, long-term program involving basic and applied research on insects and ticks which affect the health and productivity of beef cattle, horses, and swine. Studies are conducted on the biology, physiology, genetics, and nutrition of the screw-worm, stable fly, horn fly, house fly, mosquitoes and other pests; on the nature of insect resistance to insecticides; and on absorption, metabolism and excretion of insecticides by insects feeding on or in animals; the effects of irradiation and chemosterilants on insects; insect attractants and repellents; and other new approaches to control. Research is concerned with the development of more effective contact and systemic insecticides and protective treatments for the control of livestock pests. Studies are conducted to determine the occurrence of residues in tissues of animals treated with insecticides. Minor consideration is given to the development of sanitation and management procedures and biological control methods, including parasites and predators, for controlling the face fly, stable fly and several other pests. Emphasis is given to the development of insect sterility, attractants, and various other



noninsecticidal approaches to control. Studies are conducted in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions to evaluate various kinds of traps and devices for estimating and controlling natural insect populations, and improved or special equipment for the application of insecticides to animals. Limited research is conducted on the role of insects and ticks as vectors of livestock diseases, with particular emphasis on bovine anaplasmosis and equine piroplasmosis. Research is conducted at McNeese State College, the University of Southwestern Louisiana, and the University of California under contracts and at the University of Wyoming under a grant.

The Federal scientific effort devoted to research in this area totals 17.8 professional man-years. Of this number, 7.6 is devoted to basic biology, physiology and nutrition; 3.9 to insecticidal and sanitation control; 2.0 to insecticide residue determinations; 0.2 to biological control; 2.0 to insect sterility, attractants and other new approaches to control; 0.2 to the evaluation of equipment; 0.8 to insect vectors of diseases; and 1.1 to program leadership.

The Federal support devoted to research in this area under contracts and grants totals 1.2 man-years. Of this number, 0.5 is devoted to basic biology, physiology, and nutrition; 0.3 to insecticide and sanitation control, and 0.4 to biological control.

#### PROGRAM OF STATE EXPERIMENT STATIONS

Research on insects affecting livestock is an important part of the entomological program in the States. Studies on the distribution of pests and their relative importance under the varying conditions in different parts of the country form the basis for more exhaustive research on problem species. Biological information is being obtained in studies of habits, breeding sites, seasonal occurrence and dissemination. Such phenomena as host selection, feeding, behavior and host-parasite relationships are investigated by observing and collecting insects from hosts exposed in various habitats. The effects of livestock management practices on insect infestations are being determined by rotating pastures, varying forage types, and stocking rates and shelter manipulation.

Insecticides are being screened to determine their effectiveness as pest toxicants and safety. Systemic insecticides are receiving particular attention. Residue studies are being conducted to obtain basic information on the metabolism of chemicals in animals. Research is underway to determine the interrelationships in the metabolism of combinations of pesticides and to study the physiological and nutritional factors that influence the amount of tissue storage of insecticides.

Insect repellents, attractants and substances interfering with development are being identified and their effectiveness in control evaluated. Information obtained is being considered to determine the feasibility of using



such techniques in combination with chemosterilants.

A total of 21.9 professional man-years are devoted to this area by the States.

#### PROGRESS--USDA AND COOPERATIVE PROGRAM

##### A. Basic Biology, Physiology, and Nutrition

1. Mosquitoes. At Gainesville, Fla., studies were continued on the biology of Anopheles quadrimaculatus by artificially augmenting the population in an isolated breeding area. It was shown that: (1) the density of the population was increased in the study area when a constant breeding area was supplied to egg-laying females and when a blood source was provided, (2) wild females preferred natural ponds as an egg-laying site but used artificial ponds when natural areas were not present, and (3) Anopheles quadrimaculatus adults preferred resting boxes which were painted black and placed on a horizontal plane.

New rearing diets and rearing techniques were evaluated for Anopheles quadrimaculatus. Rye grass infusion or extract in the rearing medium accelerated larval development. There was a positive correlation between the number of larvae per rearing pan and larval mortality. Protozoans were an important source of food for the larvae. A rearing method incorporating all of these factors increased survival and size of the insects and decreased the time required for development and their tolerance to insecticides. Similar results were obtained with four other species of mosquitoes, Aedes aegypti, A. taeniorhynchus, Culex quinquefasciatus, and Aedes triseriatus.

A new method of separating pupae of Anopheles quadrimaculatus from larvae has been adopted. When mixtures of both are placed in ice-water, larvae sink and pupae float allowing rapid separation through the use of a funnel. Time required to separate the stages in colony production was reduced 86%.

At Corvallis, Oreg., studies were continued on the biology of the mosquito, Aedes increpitus, in the Willamette Valley. During the winter months of the last three years, larvae of this species have been collected in numerous habitats of the flood plain of the Willamette River. Following an unusually protracted period of subfreezing temperatures during which a low of 8° F was registered and near-record floods during which all low lying areas in the Willamette Valley were inundated for several days, larvae could be readily collected. The strain of increpitus in the Willamette Valley apparently has become well adapted physiologically to the rigors of the area over a long-term period of time. In other studies, a strain of Culex pipiens quinquefasciatus, which is orange in color as 4th-instar larvae and newly formed pupae, has been isolated. The strain has bred true for 3 generations and appears to be genetically recessive.

At Lake Charles, La., basic studies on the biology of floodwater mosquitoes was conducted. Studies have shown the comparative longevity, blood-feeding, and oviposition patterns of different species of flood-water Aedes species. Aedes taeniorhynchus, A. sollicitans, and A. infirmatus are more important as pest mosquitoes than other species that occur in the area. Studies on the amount of blood ingested by 12 pest mosquito species occurring in South-western Louisiana indicated that females of all species ingested sufficient blood at one feeding to at least double their body weight. Psorophora cyanescens, Aedes atlanticus-tormentor, and Anopheles quadrimaculatus, more than tripled their body weight with blood from one feeding.

Research has been conducted under two contracts at the University of South-western Louisiana and McNeese State College. Light trap collections have shown the production, relative abundance and dispersal of pest mosquitoes in the Gulf Coast area of Louisiana. Data has been maintained on rainfall and temperature in relation to mosquito production and some information has been obtained on the influence of rains versus tidal action in mosquito production. An impoundment is being developed to study the effects of impounding and water management procedures on mosquito production in the area.

2. House Fly. At Gainesville, Fla., research was continued on basic biology of the house fly. Evidence was obtained that an olfactory attractant, or pheromone, specific for the males of Musca domestica L. is not produced only by the females. The attractant was found on contaminated holding cylinders and on dead and non-virgin females. In addition, live males were also somewhat attractive. The degree of attraction was of a low order, resembling other reported sex pheromones of the house fly. This attractant was soluble in methane and slightly soluble in benzene. Data also showed that the time of day at which pupal eclosion occurs is influenced by photoperiod, but photoperiod may not be the only controlling factor. Response to insecticides was also shown to be regulated by photoperiod.

In mating experiments, female house flies mated more readily with males from their own strain than with those of other strains. When normal females from the laboratory or Grand Turk (wild) strains were confined with normal males from one strain and chemosterilized males from the other, they mated more readily with males of their own strain, whether sterilized or not. Chemo-sterilized males competed more successfully than normal males of the same strain.

At Corvallis, Oreg., research was conducted on the genetics and physiology of house flies and data developed in these studies were used to elucidate mechanisms of insect resistance to insecticides.

Several mutants of the house fly were isolated from normal and gamma-irradiated strains reared in the laboratory. Several of the mutant strains have been defined genetically and are being maintained. Most mutants involved wing form, wing positioning, or pattern of wing venation. Three established mutant strains, classic wing, stubby wing, and dot vein have



proved useful in the genetic analysis of insecticide resistance. For example, two DDT resistant strains of house flies were found to possess a fifth chromosomal dominant which confers moderate resistance to DDT, but does not confer resistance to o-chloro DDT. In addition, one of the strains possessed a second chromosomal recessive which conferred moderate resistance to DDT and high tolerance to o-chloro DDT. The presence of both factors in a strain confers virtual immunity to DDT. Substrains were isolated, each possessing only one major factor for resistance and the nature of the two independent factors for resistance confirmed through appropriate crosses and bioassays. Resistance associated with the fifth chromosomal dominant is that for DDT dehydrochlorinase. The mechanism of resistance associated with the second chromosomal recessive is unknown, but apparently does not involve dehydrochlorination.

Physiological studies of mechanisms of resistance to organophosphorus insecticides in house flies showed that blocking of ali-esterase with a selective inhibitor increased the accumulation of paraoxon and also the toxicity of parathion and paraoxon in both susceptible and parathion-resistant strains. These results indicated that ali-esterase is an important detoxifying enzyme. The role of ali-esterase in organophosphate poisoning appeared to be related more to detoxication of paraoxon than of parathion in both susceptible and parathion-resistant house flies.

A resistance factor (esterase(s)) for parathion in house flies was shown to be transmitted in a dominant manner and was at least 1000 times less sensitive to inhibition by paraoxon than a corresponding esterase present in a susceptible marker strain.

In other studies successful mating of house flies kept in complete darkness from the time of emergence from pupae to separation of the sexes occurred. Sex ratios were normal among the offspring.

3. Stable Fly. At Gainesville, Fla., studies were continued on the stable or dog fly, a serious pest of animals and humans along the northwest coast of Florida. A general outbreak of stable flies occurred during the last week of August and the first 2 weeks of September in 1964. Population counts around motels and other structures and on beaches ranged from landing rate counts of 2 to 94 flies per man per minute. Bay grass deposits in 1964 were much heavier in 1964 than in 1963. Observations indicated that the insects developed in the bay grass in about the same length of time as was required for development in CSMA medium under laboratory conditions.

Tests indicated that the effectiveness of insecticides against stable flies varied with the time of exposure after blood feeding. Other research in large outdoor cages indicated that landing rate counts of stable flies were a good index of total populations since the same percentage of flies landed on human observers when the total number of flies was varied.



In Nebraska the exposure of successive generations of stable fly larvae to DDT in the larval medium resulted in a gradual increase in tolerance to the insecticide. By the 39th generation tolerance had increased by 45-fold, but by the 42nd generation tolerance had declined to about 2-fold. The reasons for this rapid decline have not been determined.

In Texas studies were conducted to determine the effects of 4 different conditions of light (continuous light or dark, 12 hours light and 12 dark, and normal daily fluctuation) on the pattern of emergence of adult stable flies from pupae. Emergence in all tests was essentially circadian in pattern, with peaks between 6 and 9 a.m. The bulk of emergence in each group occurred on the 3rd, 4th, and 5th days after first emergence.

In Texas studies were conducted to determine the function of the antennae in feeding and mating behavior of male and female stable flies. When the antennae were completely covered with Plexiglass glue the flies did not feed and mating was greatly reduced. Additional tests showed that blinding the flies by covering the ocelli with black paint also greatly reduced mating. These results indicate that the antennae play an important role in feeding and mating responses of flies and that sight (i.e., light) is necessary for maximum mating response. Further studies showed that when stable fly pupae were held in total darkness, adults emerged and fed but only a small percentage mated and produced viable eggs.

4. Face Fly. In Nebraska, laboratory studies on the reproduction of the face fly showed that as the proportion of males to females was increased, reproduction per female decreased. This was true when only fertile males were mated with the females as well as when various ratios of fertile and sterile males were used. Decreased reproduction apparently was due to harassment by males which reduced female longevity and thereby reduced oviposition.

Studies were conducted under laboratory conditions to determine the egg-laying pattern, number and fertility of eggs and longevity of laboratory reared face flies. Female flies were allowed to mate once then confined individually in small cages for observation. Longevity of the flies ranged from 9 to 60 days, averaging 28.4 days. Batches of eggs per female ranged from 0 to 9 and the number of eggs per female varied from 0 to 181, averaging 56.6. Viability varied from 0 to 89.0%, averaging 60.2%. A few flies oviposited when 4 days old but the average age was 10 days. Results of these tests indicate that multiple matings may be necessary to insure sufficient spermatozoa to insure fertilization of all eggs laid.

Preliminary studies with 3 different strains of face flies showed very little difference in the longevity, fecundity, and rate of development of the colonies maintained under continuous light and under 16 hours of light and 8 hours of darkness. No circadian rhythms were observed but studies are being continued.

In Nebraska studies were conducted to determine the pattern and distance of dispersal of marked, laboratory reared flies released in the field. Observations indicated that the flies dispersed rapidly in all directions. Maximum distance of recovery from the point of release was 1.4 miles after 24 hours. After 10 days a few marked flies were observed at the release site.

Face flies were first observed in the field in May but populations remained low until late July, whereas in 1963 high populations developed by mid-June. Populations on cattle remained fairly constant in August but fluctuated with changes in temperature in September. When temperatures were below 70° F very few flies were active. Maximum populations of 13 per cow were noted on September 29, a warm day, but on this date flies began to hibernate in one location (grain storage building) although the inside temperature was 90° F. However, the flies moved in and out of the building and small populations persisted on cattle until the advent of cool weather in late October.

Studies were continued in 1964 on the insect fauna in cattle droppings on 3 of 8 farms studied in 1963. Over half of the insects collected in droppings were Diptera, 42% of which were face flies. The total insect population in 1964 was substantially higher than in 1963 but the number of face flies in droppings was almost identical. Parasitism of face fly pupae was low (0.7%) in 1964 but adult populations on cattle were constantly lower than in 1963. Reasons for this were not determined. In addition to face flies, droppings contained substantial numbers of aphodius beetles and Sarcophaga larvae. About 16% of Sarcophaga pupae were parasitized by Hymenoptera and Staphylinidae. Collections showed 10 species of Diptera, about 30 species of Coleoptera, and 3 species of parasitic Hymenoptera.

In Maryland, outdoor behavior of face flies was studied, both on herds in the field and on a single animal confined in a cage with a known fly population. The data indicates the following: Only a small proportion, usually less than 10-15% of the total face fly population actually annoys cattle at any given time. Female flies visit the animals much more frequently than males, but males do cause some annoyance. Three- and five-day-old females visit the cow more frequently than 1-day-old females. The number of flies present on cattle is most closely related to the activity of the animal, with the greatest annoyance when the cattle are resting quietly. Although flies visit the face most frequently, they do rest on other parts of the body in considerable numbers. An evident peak of activity of released colonized flies occurred early in the morning, but that of wild flies appeared more evenly throughout the day. All flies leave the cattle at dusk while natural light levels are still quite high.

Additional studies of the nocturnal habits of face flies confirmed that they rest on the foliage of trees at night. Although the flies are readily attracted (about 80%) to blacklight in confined spaces, those found resting on foliage at night do not respond in this manner. Electrocutor grid traps with blacklight lamps placed in trees attracted less than 1% of a released



population in 48 hours. Investigations of the factors affecting this change in behavior may provide information useful in control.

Laboratory tests of the mating activity of female face flies indicate that sterilized males compete effectively with normal males in mating. Also, females appear to mate only once if they are inseminated during their first mating. Examination of females observed attempting to remate showed that only 5-10% of all females had not received any sperm during their first mating. This characteristic indicates that use of sterilized males should be effective in preventing reproduction. A laboratory trial using a ratio of 8 sterilized males to 1 untreated male to 1 untreated female resulted in a 94% reduction in pupae.

When marked face flies were released near cows in Maryland about 1 hour before sunset, a few marked flies and a few wild flies were noted on the cows for several minutes after release. They left the cattle when the level of natural light was relatively high, indicating that artificial light would probably not be an effective attractant. In dispersal studies, 24,000 individually marked face flies were released in 4 different areas. Several marked flies were found 2 miles from their release point after 24 hours. One fly was found 4 miles away after 5 days. Searches for marked flies during the late fall showed that they spend the night in trees and tall weeds rather than in or around barns.

5. Horn Fly. In Texas a number of adult horn fly diets were compared for suitability in maintaining laboratory colonies. Diets composed of bovine blood, a saline extract of ground beef muscle and antibiotics or bovine blood, ACD, and cholesterol proved most satisfactory. Flies consumed more, reproduced better, and survived longer on these than any other diets tested, including the standard (citrate blood, tissue fluid, and antibiotics). Liquid extracts from manure of cattle fed fresh oat and dry alfalfa, sorghum, and prairie hay were poured over cotton gauze pads and compared for suitability as horn fly larval media. The oat and alfalfa extracts produced pupae that averaged 3.16 mg in weight as compared to 2.5 mg and 2.1 mg for those reared on sorghum and prairie hay extracts, respectively. In additional tests in which the pH of the media was adjusted, horn fly larvae survived and developed equally well when pH's ranged from 6.0 to 9.0 but none developed in pH's 5.5 or less or 9.5 and above.

In Texas studies showed considerable variations in the color of eggs deposited in manure by horn flies. Counts indicated about 84% of the eggs were dark brown, 13% tan, and 3% yellow and white. The eggs were equally viable and equally capable of producing progeny.

In Texas pressuring of successive generations resulted in a gradual increase in adult tolerance to ronnel. By the 20th generation a dosage of 0.1  $\mu\text{g}/\text{fly}$  caused only 32-56% mortality. By the 28th generation tests showed an LD 50 of 0.3  $\mu\text{g}/\text{fly}$  or about 50 times that of a normal susceptible strain.



6. Screw-worm. Research was continued on the screw-worm fly at Mission, Texas, in support of Southwestern screw-worm eradication program. Special studies were continued to develop a strain of flies resistant to starvation. Continuous selections resulted in a gradual increase in resistance to starvation until only negligible mortalities occurred in 72 hours in the 19th generation, in 96 hours in the 36th generation and in 144 hours in the 40th generation. Substrains removed from selection in the 18th and 30th generation retained their ability to withstand starvation. The sexual vigor of starvation resistant flies decreased gradually as resistance increased but the substrain removed from selection showed almost normal vigor 7 and 9 generations later. When females of the 27th generation of the resistant strain were held with males of the same strain under 96 hours of starvation no viable eggs were produced. When females were fed, the hatch of eggs was 38% as compared to only 14% when only males were fed. When both sexes were fed hatch was 57% or about the same as that for fed unselected females and males.

Studies were continued on the mating behavior. It has been generally believed that female screw-worm flies mate only once; however, close observations showed that a high percentage of the female flies that mated with 1-day old males (immature) mated a second time with mature males. On the other hand only 2 of 110 females first mated to mature males remated, 1 willingly and the other under duress. Egg viability was only 24% when females were mated with 1-day old males but increased to 65% when females were remated with mature males. When 1-day old males were exposed for 8 hours with 3-day old (mature) females and then replaced with mature males, egg hatch was only 52%, compared to 89% for matings of mature males and females. These results indicate that females seldom remate if their first mating is satisfactory, i.e., with a mature male.

Competitiveness of irradiated (R) and non-irradiated (N) males was compared in multiple mating of females. Frequency of remating was increased by subjecting females to starvation periods of 20 to 24 hours and all matings were confirmed by observation. Females mated first with R males and then with N males averaged 33% to 48% fertility in 2 tests, compared with 75% to 85% for the controls. However, in the reciprocal matings fertility was 80% in both tests, indicating that R males were not competitive with N males. Mortality and fecundity of starved controls subjected to single matings were normal; however, 33-71% of the remated females failed to survive. Actual cause of death following forced second matings has not been determined but these observations help to clarify SAG test results in which aggressive males greatly accelerate female mortality.

Occasionally mating and fecundity studies have been conducted in which oviposition was induced immediately after copulation. Since duration of sperm storage in some mammalian females greatly affects fertility, this possibility was investigated in screw-worms. In 2 out of 3 tests fertility ranged from 47% to 55% when oviposition occurred within 3 hours of copulation, compared with 78-86% at 24 hours. In a third test fertility was 59% within 4 minutes but ranged from 80% to 97% from 3 hours to 4 days. Although

the influence of sperm storage on fertility has not been clearly defined, a minimum 24-hour period between copulation and oviposition has been adopted in mating and fecundity studies.

Three screw-worm males selected at random from the Florida colony mated a total of 72 females each. All matings were confirmed by observation during daily 4-hour sessions. Two males ceased mating at 7 days when excessive wing damage appeared to interfere with proper positioning, and the other was dead on the 7th day. Peak mating activity occurred during the 3rd to 5th days. Total matings ranged from 1 on the 1st day to a maximum of 20 on the 3rd day; however, from the 3rd to 6th days fertility primarily occurred only among the first 7 matings. Total females fertilized per male (hatch 1% to 98%) ranged from 18 to 24. These results are in agreement with previous studies in which mating activity was evaluated only on the basis of hatching records. Although female remating seldom occurs following copulation with a mature, spermatous male (unless the females are too weak to elude the male), almost 70% of the females in the above test remated.

Studies were conducted to compare the ovarian growth of normal and starvation-resistant female flies under starvation, feeding after starvation and continuous feeding. Under starvation very little growth occurred but when food was provided the starvation-resistant females showed faster development than those of the normal strain. Ovarian growth was comparable when the two strains were fed continuously. Ovarian growth was more rapid in females fed meat than in those on a meatless diet.

Bioclimatic studies showed that both normal and selected strains of newly emerged screw-worm flies survived well when held 30 to 45 hours at 32° F, mortalities being 25% and 10%, respectively. Oviposition and egg viability of surviving females was not affected by the exposure. Exposures of 48 to 72 hours at 105° F caused 46 and 38% mortalities of the two strains and oviposition by the survivors and viability of eggs was greatly reduced.

Laboratory studies showed that male screw-worm flies reared on horse meat were about 25% larger (based on weight) than flies reared on the liquid medium now used for mass production of screw-worms for use in the Southwestern control program. Also the meat-reared males were able to mate with 40% more females than the liquid-medium reared flies, although no difference was observed in the number of mating attempts by the two strains.

A preliminary investigation made in January in Mexico indicated that in average winters there is very little screw-worm overwintering in the northwestern corner of Sonora or in the northern part of Lower California. However, in the coastal regions of Sonora between parallels 28° and 30°30', it is seldom cold enough to completely eliminate screw-worms. Instead, they are confined to certain bowl-shaped terrain features known locally as bahias. These bahias are warm and moist and furnish preferred pasture for cattle both winter and summer. It appears that the bahias have somewhat the same



relation to screw-worm survival as the river valleys in southwest Texas, but it is not known to what extent screw-worms move from one bahia to another at different times of the year.

In Texas field tests were conducted to study the relative dispersal abilities of irradiated normal and selected (starvation-resistant) strains of screw-worm flies. A total of 330,000 flies of each strain were distributed by airplane in two releases along a 6-mile swath on a large improved range area. Totals of 1659 selected strain flies and 2252 normal flies were recovered from traps, indicating that the normal flies were more vigorous than those of the selected strain.

In August 1964 a test was initiated in a 2000-square mile area in Veracruz, Mexico, to determine the efficiency of dispersal of flies dropped from aircraft at 8-mile swath intervals as compared to the standard intervals of 2-4 miles. Eleven releases of 400 males per square mile were made between August 29 and November 6. Since the area was naturally infested with screw-worms, efficiency was based on the percentage of sterile egg masses collected on wounded animals in pens located 0.1, 2 and 4 miles from release lines as compared to that on animals in a control area. Fly traps were operated at each pen from October 19 until December 1 to obtain data on the relative abundance of wild and released flies. The number of egg masses was fairly high early in August but declined rapidly with the onset of hot-dry weather and remained fairly low until late September. The numbers of egg masses began increasing with favorable weather early in October and remained fairly high until termination of the test. A few sterile egg masses were collected after the first male release. The percent sterility increased steadily thereafter to a peak of 68% by October 2 and declined gradually to about 20% at time of the last fly drop on October 27. There were no significant differences in egg mass sterility at different distances from lines of dispersal, indicating that the flies dispersed uniformly and apparently rapidly. All trap catches showed larger numbers of sterile flies than wild flies from October 26 through November 6 but native flies outnumbered sterile flies in all traps by November 14. Only 2 sterile flies were present in collections on November 19 indicating a maximum longevity of about 3 weeks.

7. Cattle Grubs. In Texas studies were continued to develop laboratory techniques for the rearing of cattle grub larvae. Several media consisting of agamma calf serum combined with various chlorides, glucose, and other materials proved highly satisfactory for in vitro rearing of cattle grub larvae. Some first instar larvae survived as long as 120 days in these media and many doubled or tripled their weight during this period. Small numbers molted to the second instar and survived another 60 days.

A major problem in in vitro rearing of cattle grub larvae is the development of bacterial infections which kill the larvae and considerable effort was devoted to the development of ways and means to solve this problem. Ultraviolet light effectively controlled bacteria but caused high mortalities



of grub larvae. Various antibiotics and merthiolate were partially effective but reduced longevity of larvae. Studies are continuing.

Studies were undertaken to determine the absorption and ingestion of food by 1st instar cattle grub larvae confined in artificial media containing P<sup>32</sup>-labeled phosphate. Examinations showed that the larvae contained much higher concentrations of radioactivity in the blood and integument than in the gut at any given time interval, indicating that most if not all intake was by absorption. Similar results were obtained with media containing dyes or carbon black. Dissections of larvae and examinations of the foreguts and hindguts failed to reveal any evidence of a lumen.

In Texas all grubs from the backs of a number of cattle imported from Wyoming were extracted and identified. The population consisted of 83% H. lineatum and 17% H. bovis.

8. Horse Flies and Deer Flies. In Mississippi, studies were conducted to develop a suitable medium for the rearing of tabanid larvae in the laboratory. Sand and soil was unsatisfactory because larvae did not survive and develop well and they could not be observed without removing them. A semi-solid agar medium proved fairly satisfactory but it tended to harden with age. Of other media tried the most satisfactory consisted of small glass beads covered with water. The larvae survived well, were able to move freely, the container and larvae were easily cleaned, and the larvae were easily observed while moving and feeding on fly larvae or worms. From 60 to 85% of larvae of I. americanus, I. abdominalis, I. proximus, and C. crepuscularis survived by this rearing technique. Survival of 4 other species was much lower. Larvae of I. vittiger schwardti showed the most rapid development from egg to mature larvae. Length of larval instars ranged from 5-6 days for the 1st, 2nd, and 3rd to 52 days for the 7th. The average time from egg to mature larvae was approximately 120 days. Attempts to mate adults were unsuccessful.

In additional tests larvae of I. vittiger schwardti developed from the 1st through the 4th stage fairly rapidly in from 5.0 to 11.3 days per stage but development of succeeding stages required successively longer times, ranging from 18.3 days for the 5th to 60.5 days for the 8th stage. Length of the pupal stage ranged from 5 to 10 days, averaging 7 days. Total days from egg to adult ranged from 143 to 209 days, averaging 189 days.

In Mississippi, after many unsuccessful attempts, a method was developed for obtaining eggs from engorged adult tabanids. Engorged adults are placed in a screen cylinder which is then inserted into a wide-mouth gallon jar containing 2-3 inches of water-saturated sand covered with a screen shield. The upper end of the screen cylinder projected 4-5 inches above the mouth of the jar and is covered with cheesecloth. This arrangement provides the range in humidity necessary to satisfy the different water requirements of various species. With this arrangement eggs were obtained from 8 species and records made of the numbers of egg masses laid and days to hatch. The

number of egg masses ranged from 1 for T. atratus and 4 other species to 10 for T. abdominalis. Eggs of T. lineola and T. vittiger schwardti hatched in 2-3 days but those of other species required from 5 to 7 days. Newly hatched larvae of most species survived and developed well in thin layers of agar in plastic dishes.

In Mississippi, adult horse fly populations increased gradually during the spring, reached a peak during late May and early June, and declined rapidly during late June and early July. Seven species were present during this period but the predominant species was T. vittiger schwardti followed by T. lineola, T. fuscicostatus, T. equalis, T. abdominalis, T. americanus and C. crepuscularis.

## B. Insecticidal and Sanitation Control

1. Mosquitoes. In Oregon 56 compounds were screened on cattle by the "spot test" method for effectiveness as toxicants and repellents against adult mosquitoes. None of the materials were outstanding toxicants. Four materials--ENT 27194, ENT 27195, ENT 27196 and ENT 28086--showed fair to good repellency at dosages of 500-1000 mg/ft<sup>2</sup>.

At Gainesville, Fla., the developmental program on insecticidal compounds for mosquitoes was continued. A large number of new candidate materials were tested in the laboratory for their potential as larvicides and adulticides. Many proved promising for further development.

Tests were conducted to evaluate fogs of naled, fenthion, Bayer 39007, and malathion against caged salt-marsh mosquito adults. Bayer 39007 was the most effective in these tests followed by fenthion, naled, and malathion. A field test in which different formulations of malathion were applied by airplane at a ratio of 0.05 lb/acre showed a reduction of 81% in population levels with the fog oil formulation, 76% with fuel-oil formulation and 44% with water emulsions. Airplane spray tests with four organophosphorus insecticides on adult salt-marsh mosquitoes indicated all were highly effective at low dosage rates. Comparison of the effectiveness of aerial sprays of malathion applied as a thermal fog and fuel oil spray showed the latter to be more effective.

Tests were conducted in the rice-growing area near Stuttgart, Ark., to evaluate the residual effectiveness of some new insecticides against natural infestations of Anopheles quadrimaculatus. The insecticides were applied to the walls and ceilings of farm buildings at 200 mg/ft<sup>2</sup> as wettable powders and/or emulsions. Pre- and post-treatment counts were made of the mosquitoes resting in the treated buildings as well as in six untreated buildings which were utilized as checks. An emulsion of Hercules 9485 was highly effective, causing 99.7% to 100% reductions for at least seven weeks. As this compound was not available at the beginning of the series, it was applied from two to three weeks after the other treatments. A wettable



powder formulation of Shell SD-8530 caused reductions of 98-100% for 9 to 10 weeks. A malathion wettable powder used as a standard caused 100% reduction for 4 weeks and 96-100% reductions (average 99.0%) throughout the 9-week test period. Hercules 9326 emulsion caused 100% reduction of the mosquito infestations for at least 9 to 10 weeks in two buildings but in a storage shed produced only 91% to 96% control the 6th and 8th weeks. Wettable powder formulations of CELA S-1942 and CELA S-2225 were slightly less effective, with control falling below 70% in some buildings by the sixth week. Shell SD-8211 was highly effective in two buildings but not in a third.

Tests were also conducted to evaluate the residual effectiveness of treated cheese-cloth when applied to the walls and ceiling of buildings in the same area. The cheese-cloth, which was purchased in rolls 3 feet wide, was first flameproofed and then impregnated with Bayer 39007. Buildings in which a complete coverage of treated cheesecloth had been used showed 100% reduction of the mosquito populations for the full 10-week duration of the test. All buildings treated by means of a strip of cloth around the edge of the ceiling and in the corners showed 100% reduction for 5 weeks, and 82 to 99% control for the next 5 weeks. Buildings treated by means of cloth around the edge of the ceiling only, or in the corners only, showed 98 to 100% reduction of mosquitoes for 5 weeks, and 84 to 100% control for the next 5 weeks.

At Corvallis, Oreg., tests were continued on the development of more effective insecticides for mosquito control. In field tests against snow-water Aedes mosquito larvae, excellent results were obtained with lindane, BHC, and fenthion at 0.05-0.1 lb/acre. Abate and Dursban were generally less effective. Against mosquito breeding in log ponds, granular formulations of fenthion and abate gave excellent control. Both were also effective when applied with a pump oil can. In cooperative tests in California low volume airplane sprays of malathion and fenthion showed considerable promise as mosquito larvicides.

Infusions and hot water extracts made from several tree species were tested for toxicity against Culex tarsalis larvae. Toxic elements were found in Western red cedar, ponderosa pine, and to a less extent in lodgepole pine and redwood. Similar hot water extracts made from Douglas fir, Sitka spruce, Western hemlock, big leaf maple, red alder, and white fir were nontoxic to larvae. Studies are in progress to characterize the toxic principles through fractionation of extracts.

None of 7 analogs of DDT showed promise against resistant Culex tarsalis larvae.

At Corvallis, Oreg., experiments with C<sup>14</sup>-TDE indicated that both susceptible and DDT-resistant Culex tarsalis larvae detoxified TDE by dehydrochlorinative and oxidative routes. The results suggested that resistance to DDT and related compounds in tarsalis involves a mechanism other than dehydrochlorination.



Studies were continued in the search for compounds that would act as synergists to overcome insecticide resistance in mosquitoes. Of a number of phosphorus esters, butyl-containing esters were most effective although other types showed activity.

2. House Fly. At Gainesville, Fla., research was conducted on the development of safer, more effective insecticides. Materials were evaluated in the laboratory as contact sprays and residual toxicants as a basis for selecting promising insecticides for field evaluations. Evaluations as residual toxicants included different formulations of the materials. Twenty-one promising compounds were tested as house fly larvicides in manure under caged poultry. Four compounds were highly effective as larvicides.

Residual tests were conducted with emulsions of malathion, diazinon, ronnel, dimethoate, naled, fenthion, and Bayer 41831, and with wettable powders of malathion and Mobil MC-A-600 against house flies in barns. All were applied at 100 mg/ft<sup>2</sup>. Control was considered satisfactory as long as the reduction produced by the chemical was 75% or above. Dimethoate residues gave satisfactory control on most occasions for 14 days, after which they were ineffective. Mobil MC-A-600 wettable powder gave satisfactory control for 14 days in one test, but failed as early as the 1st day in a replication of the test. Other compounds gave satisfactory control for shorter periods.

At Corvallis, Oreg., research was continued to find compounds effective in synergizing organophosphorus insecticides and resistant strains of house flies. A number of different types of phosphorus esters were effective when combined with either malathion or parathion in overcoming resistance in house flies to those two compounds. Materials synergizing malathion against resistant insects differed considerably from those known to potentiate the toxicity of malathion to mice or cause ataxia in poultry.

3. Stable Fly. In Texas 119 new compounds were screened in spot tests on cattle for repellency and toxicity against the stable fly. Nine of these compounds were Class IV toxicants at concentrations of 0.5% or lower. Materials effective at a low concentration of 0.1% were Shell SD-8967 and SD-9102, and CELA S-1942. The only effective repellents at a 5% concentration were ENT nos. 28086, 28087, and 28093. Special tung oil formulations from a commercial source proved both non-repellent and non-toxic to stable flies. Spot tests were conducted to evaluate a number of materials as extenders for pyrethrum and conventional insecticide. One material, Armour ARD-226, increased the repellency of malathion and extended residual effectiveness slightly. Other materials were ineffective.

In Texas, large cage tests were conducted to evaluate the effectiveness of various insecticides as pour-ons or low volume sprays for the control of stable flies. Treated cattle were exposed for 24 hours periodically to flies in large cages but otherwise were kept outdoors. Pour-ons (8 oz/animal) of coumaphos were effective against stable flies for 15 days. Pour-ons of fenthion and ronnel were only slightly less effective, but carbaryl

was effective for only 3 days. Conventional 2-quart spray applications of 0.1% Stauffer R-5723 and 1% Mobil MC-A-600 and CELA S-1942 were effective 3 to 10 days against stable flies. In similar cage tests with low volume sprays (23-69 ml/cow), 1% applications of 0.5% methoxychlor, malathion and DDT controlled stable flies for 3-6 days as compared to 1-3 days for seven other materials.

Comparative tests were run with the WHO test kit to compare the susceptibility of stable flies to 12 insecticides. Five of the materials were equal in toxicity to ronnel (standard) and 3 were more toxic, namely, Shell SD-8436, SD-8447, and SD-8448.

At Gainesville, Fla., chemicals were evaluated in the laboratory as potential larvicides for the control of stable flies. Approximately 150 compounds were tested by exposing larvae to these compounds when they were incorporated into the larval rearing medium. Approximately 20 of the compounds were highly effective, approximately equal in activity to a standard, Bayer 39007. Tests with calcium arsenate as a larvicide indicated that it did not compare favorably with other compounds that were evaluated. In addition, approximately 120 compounds were evaluated in laboratory tests as adulticides and some 20 were highly effective. Several of the more promising adulticides were tested as fogs against caged adults under field conditions, indicating the potential of these materials for controlling natural populations. Comparative tests of adulticides against caged insects indicated no differences in effectiveness of thermal vs. nonthermal fog applicators or between fuel-oil and water-based formulations. A contract was negotiated with the Florida State Board of Health to conduct research on insecticides for controlling natural populations of stable flies under conditions found in the Gulf Coast area of Northwestern Florida. The research contract will take advantage of research conducted at the Gainesville laboratory and evaluate insecticides under practical field conditions.

4. Face Fly. In Nebraska bioassay tests showed that the addition of 1.0 ppm of Thiabendazole, a new parasiticide for livestock, to manure prevented the development of face fly larvae to the adult stage. Concentrations of 0.1 ppm and lower were ineffective. The addition of 0.5% of Bacillus popilliae to manure had no effect on the development of face fly larvae but the addition of 1.0% reduced fly emergence by 24%.

5. Horn Fly. In Texas large cage tests were conducted to evaluate the effectiveness of various insecticides as pour-ons or low volume sprays for the control of horn flies. Treated cattle were exposed periodically for 24 hours to flies in large cages but otherwise were kept outdoors. Pour-ons (8 oz/animal) of coumaphos were effective against horn flies for 20 days. Pour-ons of fenthion and ronnel were slightly less effective and carbaryl was effective for only 3 days. Conventional 2-quart spray applications of 0.1% Stauffer R-5723 and 1% Mobil MC-A-600 and CELA S-1942 were effective for 7 to 10 days. In comparative tests low volume sprays of 5 and 10%



ronnel gave 100% kill of horn flies in 24 hours whereas 1% sprays gave only 86% kill.

In Oregon large cage tests were conducted to determine the effectiveness of certain tacky polybutanes against horn flies. All of the materials were repellent the first day after application but only one--Amoco H-120--showed repellency for 2 to 3 days. Additional cage tests were run to evaluate the effectiveness of 1/2 inch, 1 inch and 1 1/2 inch plastic collars containing 20% dichlorvos for the control of horn flies. The collars reduced horn fly populations by 86 to 100% in 3 hours and 100% in 24 hours and were still completely effective after 2 weeks of wear and exposure.

In the mid-coastal areas of Texas pour-on applications of 2 ounces per cow of 8% Ruelene and 5% ronnel gave excellent control of horn flies for 6 days, while applications of 4 ounces were effective for 11 days. In central Texas conventional sprays of 0.3% Ciodrin, 1% trichlorfon, 1% CELA S-1942, 0.5% carbaryl, and a pour-on of 1.0% coumaphos provided effective control for 2 weeks. Several other materials were effective for about 1 week. Similar treatments gave slightly shorter periods of control in humid coastal areas.

Low volume sprays of 5 and 10% ronnel applied to 1/ft<sup>2</sup> areas (withers and brisket) gave good control of horn flies but lower concentrations were unsatisfactory. In other tests excellent control of horn flies was obtained by treating only part of the animals in herds with 8% Ruelene at 1 oz/100 lbs body weight as a pour-on treatment. In one test the treatment of only 2 cattle in a herd of 50 reduced the overall horn fly population by 75% in 10 days.

In Texas bioassays were run to determine the toxicity to horn fly larvae of the manure from cattle that had been fed seven insecticides at varying rates for 10 days. All of the materials reduced larval survival but only Bayer 37341 and Stauffer R-3828 at 5 mg/kg daily gave 100% mortality.

In Mississippi, conventional spray applications of 2 quarts per cow of 0.375% coumaphos, 0.2% Bayer 9017, 0.5% Hooker 1422, and 0.5% methoxychlor provided effective control of horn flies for 10 days. The lowest test concentrations of 0.06% coumaphos, 0.06% trichlorfon, 0.05% Bayer 9017 and 0.1% fenthion were effective for 6 to 8 days or about as long as 2- to 4-times higher concentrations. In comparative tests back rubbers treated with 0.25, 0.5, and 1.0% Bayer 9017; 0.5 and 1.0% coumaphos; and 0.5% ronnel maintained effective control of horn flies for 7 to 8 weeks, whereas these treated with 0.5% dimethoate and 0.5% Famophos were effective only 4 weeks. Observations suggested that loss of effectiveness was due to loss of the oil solvent since the odor of insecticide was still detectable. Retreatment of each backrubber with 1 gallon of oil resulted in 4 weeks additional control, thus confirming the above observation.



In Mississippi, a series of tests were run to compare the effectiveness of 0.5% oil solutions of 10 insecticides applied in low volumes by automatic sprayers. Single applications of ronnel, Ciodrin, dioxathion, Bayer 9017, Shell Compound 4072 and Dowco 175 provided excellent control of horn flies for 6.5 to 8.5 days. Other materials were equal or slightly less effective than toxaphene (standard) which gave satisfactory control for 5 days.

6. Screw-worm. Research was continued in Texas to develop more effective insecticides for controlling screw-worms affecting livestock. Of twenty new compounds screened for larvicidal effectiveness at 10, 1.0, and 0.1 ppm in screw-worm larval medium, four were highly effective killing all the larvae at 1.0 ppm: Namely, Shell SD-8964, Shell SD-8988, Shell SD-8967, and Geigy GS-12968. None of the compounds screened were effective at 0.1 ppm.

In field tests in Mexico, cattle infested with 1- and 2-day-old screw-worm larvae were sprayed or dipped in promising insecticides. Shell Compound 4072 in a dip or spray at 0.1% killed all the larvae, as did CELA S-1942 in a 1.0% spray. Hooker HRS-1422 as a 0.25% spray and Shell Compound 4072 as a 0.08% in a dip were fairly effective but permitted a few larvae to survive.

Previous research has shown that sprays containing 0.1% or higher concentrations and a dip containing 0.1% of Shell Compound 4072 are effective screw-worm larvicides. In new tests in Mexico, cattle with wounds containing 1- and 2-day-old screw-worm larvae were dipped in vats containing either 0.05% or 0.1% Shell Compound 4072. At examination 24 hours after treatment, no live larvae were found in wounds on cattle dipped in 0.1%. All 1-day-old larvae were killed by 0.05%, and live 2-day-old larvae were found in only 1 of 16 wounds.

Telodrin as a 0.05% spray killed both 1- and 2-day-old screw-worm larvae but it also killed 3 of the 4 cattle treated. Bayer 37289 (0.25% spray) and Bayer 38333 (0.1% spray) killed all the larvae in one test, but not in another. Sprays containing 0.1% of ethion, 0.1% of Dowco 175, 0.05% of demetilan, 0.01% of Bayer 29952, 0.01% of Stauffer N-2790, or 0.01% of Bayer 38156 were ineffective.

7. Cattle Grubs and Other Bots. Research was continued in Texas and Oregon to develop more effective insecticides for the control of cattle grubs and other bots affecting livestock. In Texas 113 new compounds were screened for systemic action by giving them orally (O) or subcutaneously (SC) at several dosages to guinea pigs infested with larvae of Cochliomyia macellaria and Phormia regina. Ten materials showed systemic activity in one or both types of administration. The most effective materials, dosages, and routes of administration were as follows: Shell SD-9129, 5 mg/kg, O and SC; Spencer S-6900, 25 mg/kg, O and SC; and CELA S-2225, 25 mg/kg, O. Seven other materials were effective at dosages of 50 to 200 mg/kg.

In Texas field tests were conducted on small numbers of Government-owned cattle (2 to 4) to evaluate the effectiveness of a number of materials that had shown promise in screening tests and of several older effective materials administered in different ways at several dosages. Materials giving 91-100% control of grubs when administered in the feed for 10 days were as follows: Bayer 37341 and Bayer 37342, 1.0 mg/kg; and Famophos, menazon, and Vamidoate, 5.0 mg/kg. As drenches, CELA S-1942 at 100 mg/kg and Shell SD-8949 at 50 mg/kg gave 91 and 100% control, respectively. Menazon as a 1.0% spray gave 100% control. Other materials were ineffective.

In Texas field tests were conducted on cooperative cattle on 9 ranches with several experimental materials and with a number of older systemics administered at different dosages in several types of formulations. In pour-on tests materials, concentrations, formulations, and the lowest rates of application that gave 95-100% control were as follows: 4.0% oil suspension of coumaphos, 10 mg/kg; 15.5% water emulsion of Bayer 37342, 100 mg/kg; 4.0% water emulsion and oil suspension of Ruelene, 25 mg/kg; 7.75% water solution of trichlorfon, 50 mg/kg; and 10.2% oil suspension of ronnel, 150 mg/kg. Conventional spray treatments giving 92-100% control of grubs were 0.25% fenthion emulsion, 0.5% Rulene water suspension, 70 mg/kg; 0.25% Shell Compound 4072 water suspension, and 1.5% trichlorfon water solution, 250 mg/kg.

In Texas field tests were conducted to evaluate the effectiveness of 6 materials on Wyoming cattle infested with the northern cattle grub, Hypoderma bovis, as well as the common cattle grub, H. lineatum. Oral administration of Stauffer R-3828 at 25 mg/kg gave 90% control of grubs. The other materials were partially or completely ineffective.

In Oregon extensive field tests were conducted to evaluate the effectiveness of 7 insecticides as pour-ons and 2 as sprays for the control of cattle grubs. In these tests, pour-ons of 8% Ruelene in water emulsions at 52 mg/kg and in oil solutions at 25, 34, and 46 mg/kg per animal gave 99% control of grubs. Similar results were obtained with pour-ons of 8 and 12% trichlorfon at 30 and 45 mg/kg, and 2% fenthion at 7.5 mg/kg. Pour-ons of ENT 25482 at 91 mg/kg and Shell SD-8436 at 12 mg/kg gave 97% control of grubs and Shell SD-8447 at 121 mg/kg was only slightly less effective. Sprays of 0.1 and 0.25% Imidan<sup>®</sup> showed 88-89% control but lower concentrations were ineffective.

8. Horse Flies and Deer Flies. In Mississippi, sprays of 2% Ciodrin gave excellent immediate protection of cattle from horse flies but no effect was apparent after 3 hours. Applications of 1% Ciodrin plus 0.25% dichlorvos provided excellent protection for 7 hours and up to 24 hours in some tests.

9. Ticks. Studies were continued in Texas to develop effective systemics and conventional insecticides for use in the control of several species of ticks on cattle and other animals. A total of 114 new compounds were



screened for systemic action by giving them orally (O) or subcutaneously (SC) at several dosages to guinea pigs infested with larval lone star ticks. Only 6 of the materials showed systemic effectiveness. The outstanding materials, dosage and method of administration were as follows: Velsicol FCS-13, 25 mg/kg O and 10 mg/kg SC; and Spencer S-6900, 25 mg/kg O and SC. The other 4 materials were effective at dosages of 50-100 mg/kg by one or both methods of administration.

In Texas 160 insecticides screened in dipping tests against engorged Boophilus females to determine their effectiveness in preventing oviposition and/or hatch of eggs. A total of 44 of the insecticides were ineffective at the highest test concentration of 1.0%. The remainder were effective at 1.0% or lower concentrations. Materials that were effective at the lowest test concentration of 0.01% in preventing oviposition were as follows: Shell SD-8448 and SD-9102, and Niagara NIA-9227. Materials which permitted light oviposition but prevented hatching of eggs were as follows: carbophenothion, Stauffer R-2964, N-3727, and N-3794, Monsanto CP-40272, and Wm. Cooper 57-H-62.

Extensive field tests were conducted in Mexico to evaluate the effectiveness of 12 promising insecticides as sprays and/or dips for the control of Boophilus ticks on cattle. In dipping vat tests, concentrations of 0.05-0.1% of Shell Compound 4072 gave 100% mortality of flat and engorging stages and no live ticks were noted after 1 week, indicating that residual material killed all molting stages. Similar results were obtained with sprays of 0.1% Shell Compound 4072 and Dowco-175 and 0.01% Bayer 38333. Sprays of 0.25% Hooker HRS-1422, 0.1% ethion, 1.0% CELA S-1942, 0.25% Bayer 37289, and 0.01% Bayer 38156 were highly effective but a few ticks were still alive on treated animals after 1 week. Sprays of two materials--0.05% Teleodrin and 0.01% Bayer 29952--killed or severely poisoned cattle. Dimetilan and Stauffer N-2790 were not highly effective.

In field tests in Texas, sprays of 0.1% Shell Compound 4072 and fenthion, 0.25% Imidan and 0.5% CELA S-1942, malathion and toxaphene gave highly effective control of the winter tick, Dermacentor albipictus, on cattle. Little or no reinfestations developed on treated animals within 1 month after spraying.

In field tests in Texas, sprays of 0.25% coumaphos and Imidan, 0.03% diazinon 0.1% Shell Compound 4072, 0.3% Ciodrin, 1.0% trichlorfon, and 0.5% toxaphene gave excellent immediate control of lone star ticks on cattle. Four other materials failed to give satisfactory control. Pour-on applications of 8.0% trichlorfon and 2.0% Hercules 7522, which are excellent systemic treatments against cattle grubs, were relatively ineffective systemically against ticks.

Extensive field tests were conducted in Texas to evaluate the effectiveness of 32 insecticides as sprays and/or dusts for the control of spinose ear tick, Otobius megnini, in the ears of cattle. All of the materials except



Dri-Die and Dowco-175 dusts, and menazon sprays gave excellent to complete control of infestations of the spinose ear tick. However, the only treatments still showing effective control after 1 month were 5% dusts of coumaphos and Shell Compound 4072, 1% Hercules 7522 dust and 0.3% Ciodrin spray.

Small-scale field tests were conducted in Florida to evaluate several insecticides for effectiveness against the tropical horse tick (Derma-centor nitens), the vector of equine piroplasmosis. In these tests dermal and ear applications of 1% lindane, 0.3% Ciodrin, 0.25% Imidan and coumaphos, and 0.1% Shell Compound 4072 gave 100% immediate control of ticks but light reinfestations developed in all instances in 2 weeks. Dri-Die dust was ineffective. In systemic tests trichlorfon at 10 mg/kg a day for 10 days was completely effective in clearing ticks from the ears of horses. Fenthion at 5 mg/kg for 5 days, Famophos at 10 mg/kg for 4 days and Hercules 7522 at 5 mg/kg for 4 days reduced but did not completely eliminate tick infestations.

Surveys in southern Florida showed D. nitens to be present at 4 of 15 locations examined but populations were high in only one. Insecticidal treatment of pastures and horses apparently have eradicated the tick in the other 11 locations.

10. Lice. In Mississippi 14 promising insecticides were evaluated by the spot test method against cattle lice. Three materials, Stauffer B-10046, R-5724, and R-5725 gave 100% immediate kills of motile lice but all permitted reinfestations to develop in 14 days. In field tests, two applications of 5% dusts of coumaphos, carbaryl and dioxathion 2 weeks apart eliminated lice on cattle. Similar applications of 5% methoxychlor gave excellent control but did not completely eliminate the lice.

In Nebraska treatments of groups of cattle with 0.5% ronnel applied with a Bean Rotomist sprayer eliminated all motile stages of cattle lice. However, after 1 month light infestations were again present on some animals.

### C. Insecticide Residue Determinations

1. Residue Studies. In Texas tests were conducted to determine the levels of residues in tissues of cattle forced to use back rubbers treated with 1 and 2% ronnel-oil solutions four times daily for 28 days. Small average residues of 0.005 to 0.05 ppm were found in the fat after 2 weeks but only negligible amounts were present after 4 weeks and none whatever could be detected 2 weeks after treatments were discontinued. Residues in muscle, liver, kidney, heart, brain, and spleen were barely detectable after 2 weeks treatment and none whatever could be demonstrated thereafter.

Additional studies were conducted to determine the distribution of P<sup>32</sup> ronnel dermally on cattle forced to use backrubbers treated with this material. Comparisons were made between animals receiving 2 and 4 exposures per day for 4 weeks. Distribution as indicated by analyses of hair samples was very irregular but most of the insecticide was concentrated along and adjacent to the central back line and tip of the head. Cattle treated 4 times a day received about twice as much insecticide as those treated 2 times daily. No ronnel was present on the hair 2 weeks after the last treatments.

In Texas two tests were conducted to determine the sites of accumulation and amounts of residues in various tissues of cattle resulting from dermal sprays of 0.1% Shell Compound 4072 emulsion. In one test, the cattle were sprayed weekly for 12 weeks; in the other test they were sprayed 6 times at 2-week intervals. In the 12-weekly spray tests, analyses 1 week after the first spray showed residues in the fat ranging from 0.007 to 0.045 ppm (average 0.02 ppm). Residues increased slightly with successive weekly sprayings to a peak average of 0.14 ppm (range of 0.097 to 0.196 ppm) after the eighth spraying. One week after the twentieth and last spraying, residues in the fat averaged only 0.01 ppm (range 0.008 to 0.016 ppm). No residues were detectable 2 weeks after the last spraying. In the biweekly spray test analyses 2 weeks after the first and third sprays showed only 0.005 ppm in the fat but residues increased somewhat after the fifth and sixth sprays, averaging 0.117 and 0.133 ppm, respectively. Additional analyses of fat from animals slaughtered 2 weeks after the sixth and last spraying showed average residues of 0.065 and 0.112 ppm in renal and omental fat, respectively. No residues were detectable 4 weeks after the last spraying.

Analyses of tissues from a calf slaughtered 7 weeks after being sprayed with 0.25% Imidan showed no residues in samples of fat, muscle, heart, liver or spleen.

In Maryland, analyses were made to determine the levels of residues in fat samples of swine 1, 4, 7, 14, 21, and 28 days after treatment with 0.05% lindane emulsion. Maximum residues were indicated 4 to 7 days after treatment but declined steadily thereafter. Four weeks after treatment only 0.1-0.3 ppm was present in the swine fat.

In Maryland, as a result of the detection of residues of heptachlor epoxide in the fat of cattle slaughtered at the Agricultural Research Center at Beltsville, analyses were made to determine if residues were present in the milk of three experimental dairy herds. The over-all average residue of heptachlor epoxide in the milk was below 0.01ppm. Analyses of eight lots of hay and one lot of alfalfa pellets which were being fed to the cattle showed residues ranging from 0.00 to 0.048 ppm of heptachlor epoxide. Subsequent tests did not show detectable residues in the feed but low levels of heptachlor epoxide persisted in the milk of selected animals from experimental herds.



2. Toxicity Studies. Research was continued in Texas in cooperation with veterinarians of the Animal Disease and Parasite Research Division on the acute and chronic toxicity of insecticides and other chemicals to livestock.

Studies were conducted to determine the normal patterns of certain enzymes in average cattle as a prerequisite for studying the effects of chemical poisoning on enzyme patterns and the effects of oxime-type cholinesterase reactivators (2-PAM, DAM and TMB-4). Cattle poisoned by Dioxathion caused elevations in activity of serum glutamic oxalate, pyruvate transminases, alkaline phosphatase, and blood beta lipoprotein but these increases were minimized by the administration of 2-PAM and TMB-4. All three test oximes prevented decreases in gamma globulin. These results indicated that TMB-4 was slightly more beneficial than 2-PAM. DAM did not appear beneficial at the levels (10-20 mg/kg) tested.

Cattle were poisoned with an oral dosage of dichlorvos to determine the effects on serum glutamic oxalacetic and pyruvic transminase, aldolase and alkaline phosphatase. Oximes were given some of the cattle to determine their protection of those enzyme systemics: DAM and 2-PAM kept the enzyme activities of the mildly poisoned animals near normal, whereas TMB-4 appeared to cause an increase in activity above normal during the test. From the biochemical standpoint, it appeared that 2-PAM and DAM offered more protection to the enzyme activities than does TMB-4.

Cattle were poisoned with coumaphos and enzyme systems studied in serum. Some of the cattle received antidotal therapy with 2-PAM. Glutamic dehydrogenase, sorbital dehydrogenase, phosphohexose isomerase and serum arginase were studied in an effort to find significant enzyme activity alternations indicative of possible tissue change. No significant differences were noticed in the enzyme activities regardless of treatment. Mortality was reduced by 2-PAM in coumaphos poisoned animals, but this benefit could not be detected in the enzyme studies.

Additional studies were conducted to determine the effectiveness of these three oximes in reversing cholinesterase inhibition induced by organic phosphorus compounds. Each of the compounds was useful, but 2-PAM and TMB-4 appeared to be superior to DAM. Particularly encouraging was the beneficial effect of these compounds in cattle poisoned by coumaphos; usually such animals do not readily respond to atropine, the pharmacologic antidote. Oximes combined with atropine markedly increased the number and speed of recoveries.

Studies were conducted to determine the effects of the chemosterilant, apholate, when fed to Jersey cattle at 1 mg/kg daily throughout one gestation period. A deficiency of white blood cells appeared in one heifer after 80 daily doses and in the others after 110. One heifer died after 335 doses, one delivered a calf and died after 531 doses, one delivered prematurely and survived 581 doses, and the fourth delivered at term and survived 629 doses. In additional studies the new chemosterilant, hempa, appeared to be



considerably less toxic than apholate, tepa and metepa, but it produced the same deficiency in white blood cells.

Studies were conducted to determine the toxicity of 72 insecticides to cattle, sheep and goats. Toxicities ranged from impossibly dangerous to reasonably safe. Promising new insecticides showing low toxicities were Shell SD-8447 and CELA S-1942.

#### D. Biological Control

1. Mosquitoes. At Lake Charles, La., research on pathogens of mosquitoes has been conducted. Field collections throughout the area have shown infections of microsporidia in thirteen species including the genera, Culex, Aedes, Culiseta, Anopheles, and Orthopodomyia. Aedes grossbecki and Orthopodomyia signifera were new host records for a microsporidian. Spore sizes were determined and classification of the microsporidia studied. Transovarial transmission of microsporidian infections was studied in 12 species of mosquitoes and demonstrated in seven of the species.

The fungus Coelomomyces was found in field collections infecting larvae of Culex restuans, C. salinarius, Aedes vexans, A. sollicitans and Culiseta inornata. Culex salinarius, C. restuans and Aedes sollicitans represent New host records for Coelomomyces. Field infection levels varied from very low to over 50%.

A polyhedral virus was reported from larvae of Aedes vexans and Psorophora ferox. Both species were previously unreported as hosts of viruses. Infection levels in the field were very low. A very lethal bacteria was collected from larvae of six mosquito species.

At Gainesville, Fla., two species of Thelohania were found in A. quadrimaculatus, one infecting the adipose tissue and the other the oenocytes. One species was found infecting the oenocytes of A. crucians larvae.

2. Face Fly. Studies in 1963-64 by personnel of the European Parasite Laboratory, Insect Identification and Parasite Introduction Research Branch, in France showed that the adults of Aelochara tristis, a staphylinid beetle, were predaceous on larvae of the face fly and other Diptera breeding in cattle droppings and that newly hatched larvae parasitize face fly pupae. Shipments of this species were received at Lincoln, Nebr., in January and February 1965 and immediate steps were taken to establish colonies. The adult beetles were caged under room conditions (80-85° F; 50-60% RH) and provided fresh cattle manure containing face fly eggs and small larvae. The beetles survived well, oviposited and newly hatched larvae were noted in 12 days. Face fly pupae were made available to the small larvae. Observations showed that the larvae quickly penetrated the face fly puparia and closed the entrance hole. The larvae consumed the face fly pupae and pupated within the puparia. Adult beetles emerged from the puparia through the area

normally used by emerging flies. The combined larvae and pupal period was about 17 days and the adult preoviposition period of about 12 days. Large numbers of adults will be produced and released at selected field sites near Lincoln and studies made to determine the effectiveness of this parasite in reducing face fly populations.

3. Horse Flies and Deer Flies. In Mississippi, large numbers of tabanid larvae were collected periodically and transported to the Kerrville, Texas, laboratory to determine the number and species parasitized and the identity of the parasites. A total of 206 larvae, representing 4 species, were collected in October 1964. Only 3 larvae of T. atratus showed microsporidian infections. In subsequent collections in November 1964 and January 1965, microsporidian infections were found in some of 3 species of tabanid larvae, namely, T. sulcifrans, T. vittiger schwartzi, and T. atratus. The microsporidia in sulfifrans could not be identified but those infecting vittiger schwartzi were of the genus Plistophora. Those infecting atratus were Thelohania sp. Healthy atratus larvae readily became infected when fed spores of Thelohania but not when fed those of Plistophora. Microsporidian infections could not be induced in fed or starved healthy larvae held at 10° C. At 25° C, fed larvae readily developed infections but not starved larvae.

In Texas, approximately 300 tabanid larvae were collected and examined during the year and all were free of microsporidia and other parasitic infections.

Studies were conducted to determine the host range of microsporidia (Thelohania sp.) by feeding artificially infected C. macellaria larvae to different species of tabanid larvae. The normal host of this parasite, Tabanus atratus, readily became infected. Of 5 other species used, only one, T. americanus, developed typical spore infections.

Similar studies were made with another microsporidian (Plistophora) but results were negative.

In Texas efforts to develop a reliable spore agglutination test based on sedimentation patterns formed by spores mixed and allowed to settle with rabbit antisera were unsuccessful. In other tests antimicrosporidia spore-rabbit serum reacted positively by agglutination against homologous antigen but further tests must be conducted to ascertain the specificity of the reaction.

4. Ticks. In Texas laboratory tests were conducted to determine the effectiveness of a dust preparation of the fungus Beauveria bassiana against 4 species of ticks. Adults and nymphs of Amblyomma americanum, adults of A. maculatum and adults of Dermacentor variabilis were highly susceptible to the fungus with 90 to 100% kills occurring in 7 to 10 days. Nymphs of the spinose ear tick, Otobius magnini, were not affected.



## E. Insect Sterility, Attractants, and Other New Approaches to Control

1. Mosquitoes. At Gainesville, Fla., studies were continued on factors affecting the attraction of mosquitoes to their hosts and factors affecting the protection time from mosquito bites afforded by repellents. A large olfactometer was developed to study these factors as well as evaluate the efficacy of various attractant materials or factors.

The effort to develop effective space and systemic repellents was continued and slightly expanded. To date several materials show some space repellency to mosquitoes in that they prevent mosquitoes from penetrating 4-mesh screening. Materials exhibiting some systemic repellency were found and further tests will be made on these materials.

Studies were continued at Gainesville to evaluate materials as chemosterilants for mosquitoes and to evaluate the sterility principle of mosquito control. Tests with hempa indicated it would have little value as a residual sterilant for mosquitoes. Further selection and studies were conducted with the apholate-resistant colony of Aedes aegypti to clearly define the degree of resistance. This colony is at least 10 times as resistant to the sterilizing effects of apholate as the unselected, parent colony. Selections to increase resistance will be continued.

A sterile-male release study of Anopheles quadrimaculatus was made in a semi-isolated area. This site was made more favorable by increasing the number of breeding sites and introducing additional wild stock of this mosquito to populate the area. When sterile males of wild stock were released in this area, sterility of the natural population increased from a very low degree up to 42%. When the releases were changed to sterile colony males the sterility in the natural population decreased confirming earlier work showing behavior differences between colony and wild strains in seeking out wild females. Apparently a sufficient number of males was not released to reduce the population levels of A. quadrimaculatus.

Studies were continued on the evaluation of chemosterilants for mosquitoes at Corvallis, Oreg. The chemosterilant, hempa, was not highly effective in sterilizing Culex tarsalis larvae in that rates as high as 200 ppm were required. As a residual treatment in glass jars, 10 mg of hempa per square foot sterilized adult males completely, but adult females only partially. In wind tunnel tests against adults, a concentration of 10% caused high sterility, whereas a 5% spray caused only partial sterility and none was caused at 1% or lower. Males were generally more susceptible than the females.

At Corvallis studies were continued on sex and ovipositional attractants for mosquitoes. Preliminary tests indicated the presence of a sex attractant in Culex quinquefasciatus, but further tests did not confirm its presence, nor the presence of a sex attractant in Culex tarsalis. Many mosquitoes are known to choose specific types of water for oviposition. Studies have shown



that odors from grass infusions and log pond waters collected in distilled water were attractive to gravid females of Culex quinquefasciatus. These odors were not attractive to females of C. tarsalis. However, log pond water itself was more attractive to this species than either distilled water or distilled water plus log pond odors. Distilled water saturated with methane was also attractive to gravid females of C. quinquefasciatus but not to those of C. tarsalis. Gravid females of both species were more attracted to distilled water treated with 25 ppm of furfural than to water treated with 5 or 50 ppm.

2. House Flies. At Gainesville, Fla., research was continued on the development of chemosterilants and the sterility principle of control for house flies. Several hundred new candidate compounds were evaluated in primary screening and secondary development tests and many were found to exhibit sterilizing efficacy against both males and females. Particular attention was paid to evaluating two compounds--hempa and hemel--as sterilants by several routes of administration. These two materials will sterilize both sexes of the house fly.

Two series of field tests were conducted at farms in Florida to evaluate the effectiveness of two chemosterilants, hempa and apholate, for the control of house flies. At the farm treated with hempa, house flies were reduced in abundance from 71 per grid to 0 within eight weeks. Grid counts remained at zero for the remainder of the test period. At the farm treated with apholate population levels decreased from 200 per grid to less than 10 within 6 weeks and remained constant at a low level throughout the remainder of the test.

Ninety chemicals were screened as chemosterilants against adult house flies. Six compounds were toxic and 10 reduced the fertility to some extent in fly food or sugar. Thirty-two compounds previously shown to sterilize house flies were also tested again at higher or lower concentrations.

Tests were conducted with 21 compounds to determine their effectiveness as male house fly sterilants. Of the 14 chemicals Olin 53330, Squibb Olin 53331, Squibb Olin 53356, and Squibb Olin 53263 sterilized at a concentration of 0.05% in the sugar diet. Sankyo Co. RES-101 induced sterility at this dosage in sugar in the first two eggings.

Basic studies were continued on the cytological effects of chemosterilants on house fly reproductive systems and previous sectioning, fixing, and staining techniques have been used to study several new chemosterilants.

Olfactometers designed by Gouck and Schreck were used to initiate a search for more effective house fly attractants. Nineteen compounds were tested as house fly attractants, using Edamin as a standard. Beef protein concentrate was attractive to females but not to males. The other materials were not as effective as Edamin.

At Corvallis, Oreg., research was conducted on chemosterilants and attractants for the house fly and the little house fly. With the little house fly, hempa caused sterility as a residual deposit on glass at 50 mg/ft<sup>2</sup>. However, the sterilizing dose caused some fly mortality. Higher doses were highly toxic to the adult flies; lower doses did not sterilize. When fed orally in the adult food to the little house fly, hempa was toxic at 0.25% and lethal at 1.0%. High, but incomplete sterility was caused at concentrations as low as 0.01%. Topical treatments of hempa and hemel sterilized males of the little house fly without causing mortality, but not the females. In general treatments causing a high degree of sterility did not affect the mating competitiveness of females. Four known antioxidants exhibited little effect on egg production or egg hatch with the little house fly. Dosages of gamma radiation greater than 1000 r given to pupae of the little house fly prevented oviposition by emerging adults.

At Corvallis research was continued on the sex pheromone in house flies. The presence of a low titre of pheromone in extracts of pupae and young females was shown and confirmed. Higher activity of the pheromone was demonstrated in 3-day-old flies. The presence of the pheromone was shown in female flies from strains of different origin. Males of different strains reacted to extracts from females of different strains, though differences in behavior were apparent.

At Corvallis, Oreg., further studies showed that the sex pheromone in female house flies increased with the age of the flies, with the greatest increase occurring on about the third day after emergence. Comparative tests with benzene extracts of female house flies showed that high concentrations applied to pseudo flies inhibited male response. Similar inhibition occurred when normal extracts were applied to large surfaces within test chambers. Comparative tests with extracts of females with different solvents showed a much greater amount of the pheromone in hexane than in other solvent extracts. Efforts are being made to determine the chemical nature of the pheromone.

At Beltsville, Md., the effectiveness of electrocutor-grid screens placed in window plus an indoor electrocutor trap with black light lamps for controlling fly populations was evaluated in two calf barns where large numbers of house flies were present. Although many flies were killed by the grids, no substantial reduction of the fly population within the grid-screened barn could be measured unless all major openings were closed or screened. When the animals were allowed access to exercise lots through open doors, the fly populations in the test barn and the "check" rapidly equalized.

3. Stable Fly. At Beltsville, Md., the effectiveness of electrocutor-grid screens placed in window plus an indoor electrocutor trap with black light lamps for controlling fly populations was evaluated in two calf barns where large numbers of stable flies were present. Although many flies were killed by the grids, no substantial reduction of the fly population within the grid-screened barn could be measured unless all major openings were closed or



screened. When the animals were allowed access to exercise lots through open doors, the fly populations in the test barn and the "check" rapidly equalized.

4. Face Fly. At Beltsville, Md., additional tests of the responses of 3-day-old female face flies to monochromatic light confirmed that blacklight is highly attractive under conditions of confinement and that wavelengths in the red and yellow spectral regions are unattractive. Refinements of the testing procedure and methods of data analysis are being made in an attempt to more clearly establish differences in attractiveness.

5. Horn Fly. In Texas, topical applications of 1  $\mu\text{g}/\text{fly}$  of apholate sterilized adult horn flies. Lower dosages of 0.5, 0.2 and 0.1  $\mu\text{g}/\text{fly}$  reduced the hatch of eggs but did not confer complete sterility. Males were more easily sterilized than females. In feeding tests, flies feeding overnight on a diet containing as little as 0.01% apholate were completely sterilized.

6. Screw-worm. In Texas 20 of 255 compounds screened as chemosterilants caused sterility in one or both sexes of screw-worms when administered as topical treatments or fed to adult screw-worm flies. Some of the compounds sterilized by both methods of administration, some sterilized only one sex, and some sterilized both male and female flies. A review of past chemosterilant screening revealed that of the aziridine compounds screened, 92 were effective either by multiple-oral administration or topical application, or both. Only 8 were less than 100% effective when administered orally. In the future, routine use of topical applications in chemosterilant screening will be dropped in favor of multiple-oral administration.

It has been shown that there is a differential susceptibility between males and females sterilized with metepa. Tests with uredepa (ENT-50450) showed similar results, with the males about 9 times more susceptible than females on the basis of dosage/unit of body weight. There was also a greater variation in results obtained with females than with males. Starvation also increased the effectiveness of uredepa. The antifertility effects of another chemosterilant, ENT-25296, were enhanced by subjecting treated flies to periods of temperature stress (98° or 140° F) after administration of sterilizing or highly effective substerilizing dosages.

Male and female screw-worm flies can be sterilized by exposure to certain chemosterilants, but most of these chemicals adversely affect mating activity, longevity, or vigor. Three new chemosterilants were found that equal or surpass radiation in their effectiveness in achieving sterility of screw-worms. ENT-50838 applied topically provided a wide margin of safety between the minimum toxic quantity and the sterilizing dosage. Males sterilized with this material were hypercompetitive; they were sexually more aggressive than irradiated flies. The other two compounds, ENT-50716 and ENT-50781, were more toxic to the flies, but they were approximately equal to radiation as sterilizing agents.



In Texas, 154 chemicals and other materials were screened as screw-worm attractants. Of these, 22 were equal to or better than the standard liver bait and require further evaluation. Some have been tested in the field in limited tests. The most outstanding were isovaleraldehyde, ethyl isovalerate, and an ethanol extract of the flowers of Yucca treculeana. Liver-baited traps were seldom as effective as traps containing these materials. The presence of blooming wild flowers interfered with these tests; tests made during peak blooming seasons usually gave negative results.

The presence of a pheromone produced by males that is attractive to virgin female screw-worm flies was confirmed. In Texas, by means of the cold-trap method, 3500 ml of condensate were collected over a 5 1/2-month period from a cage containing virgin male screw-worm flies. Benzene and chloroform extracts of the condensate were capable of changing the behavioral pattern of virgin female flies. Sexually mature females in the presence of the odor go through "searching" motions and finally behave as in an aggressive mating "strike." Young (1-2 days old) females gave little or no response to the male odor, but 3-day-old females exhibited a definite activity, including the imitation of "male-type strike." Four-day-old females reverted to the response of 1-day-old females, but activity increased again in 5-day-old females. The greatest response was observed in 6-day-old females, with 7- and 8-day-old females showing a decrease in total number of "strikes." This decrease may have been due to wing damage, normal at this age. Other studies are in progress, including fractionation of the extracts to find the effective material. Another extract, made by filtering the air in the eradication colony room, brought a response on the part of both males and females.

7. Ticks. In Texas, studies on the effects of radiation on lone star ticks showed that dosages of 250 and 500 r did not affect fertility of adults treated 1 week after molting from nymphs. However, a dose of 500 r sterilized females 1 day after engorging. Doses of 250 and 500 r had no effect on engorging and molting of nymphs or on fertility of resulting adults. However, at 1000 r the percentage molting of nymphs was reduced and resulting adults did not engorge or lay viable eggs.

#### F. Insect Vectors of Diseases

1. Anaplasmosis. Studies were continued in Texas and Mississippi in co-operation with the Animal Disease and Parasite Research Division and veterinarians of the State Experiment Stations to correlate the presence and abundance of insects and ticks with the incidence of anaplasmosis in herds of cattle. Texas, monthly surveys were made to determine the identity and abundance of external parasites on infected and clean (segregated) herds of

cattle. Lone star tick populations were light on cattle in January, increased gradually during February, March and April, were heavy from May through July and then declined rapidly to insignificant numbers by September. Winter ticks first appeared late in October, increased gradually to peak abundance in late December, then declined to insignificance by March. The spinose ear tick was present throughout the year, with populations being moderate to high at practically every examination. Cattle lice populations were extremely low throughout the year. Horn flies appeared in April, increased steadily to moderate numbers by June, remained at this level until late September, and disappeared with cool weather during late October and early November. Light to moderate grub infestations were present in the backs of cattle from early January until mid-March. Some transmission continued to occur in the infected herd but the segregated herd remained clean. It was thus demonstrated that young calves removed from the presence of carrier cows could be maintained anaplasmosis-free. The test was discontinued this year.

In Mississippi, studies on the relative importance of day- and night-feeding insects in the transmission of anaplasmosis, which were initiated in 1963, were continued in 1964. In the main test, three groups containing negative, splenectomized steers were used, one group being exposed continuously, one during the day only, and one at night only. The test period of 6 weeks (June 1 - July 13) was divided into three 2-week intervals and during each interval one other group of cattle of the same composition was exposed continuously in hopes of pinpointing the 2-week period in which most transmission occurred. Each group was exposed daily with animals infested with anaplasmosis.

During the first two weeks (June 1-13) horse flies were moderately abundant, but declined in numbers abruptly and only small numbers were present the last 3 weeks of the test. Populations of horn flies were relatively low and stable flies relatively high throughout the test period. Mosquitoes were fairly numerous the first week of the test but populations declined thereafter and remained low throughout June. Populations rose rapidly early in July and were high during the last two weeks of the test. The mosquito population was largely Psorophora confinnis until the last week of the test, when Anopheles quadrimaculatus increased abruptly to make up about 40% of the population.

In the main 6-weeks test, two cases of anaplasmosis developed in each of the continuous- and day-exposure groups, but none occurred in the night-exposure group. In the other groups exposed continuously for 2 weeks, one case each occurred in the first (June 1-16) and second (June 16-29) exposure periods but none developed in the third (June 29-July 13). These results indicate that anaplasmosis transmission most likely occurred during June when horse flies were most abundant. This indication is supported by the fact that no transmission occurred in the cattle exposed at night when only mosquitoes attacked them. However, additional studies are needed to clarify



the role of mosquitoes as possible vectors and to determine whether one or more species of horse flies is capable of transmitting the disease.

At Beltsville, Md., studies on the transmission of bovine anaplasmosis and the development of the disease organism in experimental vectors were continued in cooperation with personnel of the Animal Disease and Parasite Research Division.

Efforts to colonize a "Nevada line" of Dermacentor andersoni were unsuccessful. The larval progeny from a single engorged female received from Nevada did not attach and feed on the test calf, and all died.

An adult-to-larva hereditary transmission trial with Dermacentor occidentalis was negative. Several thousand larvae attached and fed on the test calf, but anaplasmosis was not transmitted. The calf was proved susceptible by inoculation with Anaplasma infected blood.

Studies were conducted with Dermacentor occidentalis to determine whether or not the ticks can become adapted to infection with Anaplasma marginale by feeding one or two of the developmental stages in each generation on calves acutely infected with A. marginale. The plan of study was to feed adults on infected calves, feed the resulting larval progeny on guinea pigs and then test part of the ensuing nymphs on a susceptible calf. If transmission did not occur, the remaining nymphs and succeeding adults were to be fed on acutely infected calves and tested again in the following generation. In the first generation studies, the adult-to-nymph trial was negative and the test calf proved susceptible by challenge. Approximately 1,000 nymphs were allowed to feed on an infected calf and 50 of the resulting adults were tested on a susceptible calf for a trans-stadial transmission. This experiment has not yet been concluded. The remainder of the D. occidentalis adults, infected as nymphs will be fed again on an infected calf and tested again as 2nd generation nymphs.

Systematic studies on Anaplasma-infected and non-infected D. occidentalis salivary glands are being conducted concurrently with each transmission experiment.

2. Equine Piroplasmiasis. During the last year at Beltsville, Md., larval progeny of field collected D. nitens adults from two Florida sources were tested on susceptible horses to determine their infectivity status. Transmission did not occur and subsequently both test horses were shown to be susceptible by inoculations of Babesia caballi infected blood.

A transmission trial with the larval progeny of D. nitens adults collected in Puerto Rico was also conducted with negative results. This test horse was also subsequently shown to be susceptible by inoculation of Babesia caballi infected blood.



Transmission experiments are now under way with the larval progeny of D. nitens adults collected from infected horses in Florida during June 1965. Results of these tests are not yet available.

Cytological studies on D. nitens were conducted to gain a better understanding of the disease agent and its relationship to the vector. All stages of D. nitens were taken from horses infected with Babesia caballi and prepared for histological study. Structures believed to be developing forms of Babesia caballi were observed in sections of some specimens.

Another and more virulent strain of Piroplasmosis, Babesia equi, was found for the first time in Florida this year. The vector of this disease has not been determined as yet but transmission studies with several species of ticks and other external parasites are planned.

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## LIVESTOCK ENGINEERING

Agricultural Engineering Research Division, ARS

Problem. Modern production trends are demanding more basic knowledge about the effects of environment on the health, growth, production and fertility of livestock; about structures and related equipment for maintaining desirable environments; and about methods, structures and equipment for more efficient handling and feeding. The continuing threat of nuclear warfare demands consideration of types of buildings that will provide protection from fallout for livestock and their feeds, and provide facilities for operation during periods of emergency.

Closely associated with the environment are flies and other insects, as well as parasites and diseases, that sap the vitality of animals and reduce their productivity. Pesticide residues in animal products are causing much concern. Information is needed on means for keeping these residues from adversely affecting the animals or their products. How to adapt existing buildings and other facilities for more efficient production, as herds and flocks are increased in size, or as farms are consolidated, is a major problem area. Adaptations and improvements are needed to keep design of such equipment as feed bunks, self-feeding silos, and feeding floors abreast of current production practices.

An adequate supply of satisfactory water is essential to the farmstead. Disposal of organic wastes--principally sewage and manures--is becoming more and more of a problem on the modern farmstead. The problem is particularly acute with respect to confinement-type livestock operations on the fringes of metropolitan areas--where the total amount of manure is concentrated in the confinement area. Under these conditions, it is difficult to avoid creating a sanitation hazard or a public nuisance. Economical, sanitary means of disposition need to be developed. The arrangement plan of the farmstead has an important bearing on its efficiency, appearance, and livability.

## USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving agricultural engineers, architects, electrical engineers, and physicists, engaged in basic and applied research in cooperation with representatives of other research disciplines. Much of the work is conducted cooperatively with State Experiment Stations and industry. The program is conducted and reported under the following categories:

A. Dairy Cattle Engineering. Dairy cattle environmental and bio-engineering studies are conducted in a climatic laboratory at Columbia, Missouri, in cooperation with the Dairy Husbandry and Agricultural Engineering Departments of the Missouri Station. AH, ARS, serves in an advisory capacity. The influences of building arrangement, equipment, and chore routines on the amount and drudgery of dairy chores and means of improving these factors are studied in cooperation with the California Agricultural Experiment Station. Typical plans for dairy structures are developed at Beltsville, Maryland.

B. Beef Cattle Engineering. Beef cattle structures and equipment research for hot, dry climates is conducted in cooperation with the California Agricultural Experiment Station at the Imperial Valley Field Station, El Centro. Typical plans for beef structures are developed at Beltsville, Maryland.

C. Swine Engineering. Swine structures and equipment research for hot, dry climates is in cooperation with the California Agricultural Experiment Station at Davis and for hot, humid regions at Tifton, Georgia; in cooperation with the Georgia Coastal Plain Experiment Station and AH, ARS, on an "occasional visit" basis. Typical plans for swine structures are developed at Beltsville, Maryland.

D. Poultry Engineering. Poultry house environmental design criteria are investigated in controlled-temperature laboratory studies at Beltsville, Maryland, in cooperation with AH, ARS, and the basic laboratory data are applied to experimental poultry houses of the NE-8 Regional Project for evaluation.

Field studies on relation of housing structures to poultry disease are conducted in Mississippi in cooperation with the State Agricultural Experiment Station and AH, ARS. Environmental influences on health and housing requirements are investigated in new laboratories at Athens, Georgia, and State College, Mississippi, in cooperation with AH and ADP, ARS, and the respective State Agricultural Experiment Stations. At St. Paul, Minnesota, a study of the role of environment in the prevention and control of chronic respiratory disease in turkeys is underway in cooperation with the Minnesota Agricultural Experiment Station. Typical plans for poultry structures are developed at Beltsville, Maryland.

E. Livestock Shades and Shelters. Shades for sheltering livestock are being studied at Davis, California and Tifton, Georgia, in cooperation with the respective State Agricultural Experiment Stations.

F. Sky Radiosity Studies. Studies of sky radiosity (total radiation) are conducted at Davis and elsewhere in California, and at Columbia, Missouri, in cooperation with the respective Agricultural Experiment Stations.



G. Reducing Pesticide Residues in Animal Products. Reduction of pesticide residues in animal products, with beef cattle receiving major attention, is studied at Kerrville, Texas, in cooperation with ENT and ADP, ARS and the Texas Agricultural Experiment Station.

H. Water Supply and Wastes Disposal for the farmstead are studied at College Park, Maryland, in cooperation with the Maryland Agricultural Experiment Station. Liaison is maintained with the Public Health Service, the Water Systems Council, the American Society of Agricultural Engineers, and other organizations concerned with rural sanitation.

I. Farmstead Planning studies are made at Beltsville, Maryland, at St. Paul, Minnesota, in cooperation with the Minnesota Agricultural Experiment Station, and at Davis, California, in cooperation with the California Agricultural Experiment Station.

J. Fly Control in dairy barns is studied at Beltsville, Maryland.

K. Effects of Electric Glow-discharge Radiation on Salmonella were studied at Pullman, Washington.

L. Equipment and Control for Automatic Feeding of livestock and poultry is under development at the Washington and Illinois State Experiment Stations. Work on performance characteristics of upright-silo unloaders is in cooperation with the Minnesota State Experiment Station.

M. Electric and Solar Equipment for Environmental Control. Research on equipment for basic and applied studies involving light and thermal environment for poultry is underway at Beltsville in cooperation with the Poultry Branch, Animal Husbandry Research Division. Basic and applied studies on the use of heat pumps to modify thermal environment for hog production is conducted at Holland, Virginia, in cooperation with the Virginia Agricultural Experiment Station.

The influence of electric equipment and environment on health and disease is being studied in USDA laboratories at Athens, Georgia. Studies on the performance of milk handling equipment are underway at Beltsville in cooperation with the Animal Husbandry Research Division and the Eastern Utilization Laboratory.

The Federal research effort devoted to this area amounts to 18.9 professional man-years of which 2.3 are devoted to dairy structures and equipment; 0.8 to beef structures and equipment; 1.2 to swine structures and equipment; 3.4 to poultry structures and equipment; 0.4 to sky radioactivity studies; 1.0 to reducing pesticide residues in animal products; 2.4 to water supply and waste disposal; 1.0 to farmstead planning; 2.0 to physical methods of fly control; 0.1 to electrical glow-discharge radiation

on Salmonella; 2.0 to electrical equipment for livestock and poultry; 1.6 to poultry environment equipment; 0.2 to swine environment equipment; and 0.5 to milk cooling equipment.

#### PROGRAM OF STATE EXPERIMENT STATIONS

There is an extensive program of both basic and applied research underway at the State Agricultural Experiment Stations in an effort to provide the answers to the continuing series of questions being raised by livestock producers. Demands are being made for more information on the effects of environment on the physical well-being of all classes of livestock, and the way such optimum environments can be economically achieved; on new approaches to meet the growing labor shortage; on methods to adapt existing structures and equipment for greater economy of production; and on structures and related equipment for improved efficiency of feeding and materials handling operations.

Studies are being made on the effect of environment on the health, growth, production and fertility of dairy cattle, beef cattle, poultry and swine. Equipment and systems for efficiently transporting feedstuff into and out of storages and automatically mixing and feeding complete rations are being developed.

Exploring methods for improved care and housing of farm animals with greater economy and labor efficiency are also in progress as well as investigation of ways to modify existing structures and equipment to meet present-day economic conditions.

In the water supply area research is underway to develop ways to economically filter and treat surface waters in order to provide an adequate and sanitary quantity of water for the farmstead operations. Studies are also being made on the problems concerned with demineralizing deeper ground waters.

A widespread research effort is in progress which is attempting to investigate all of the factors involved in the complicated problems concerned with disposal of farm waste materials, particularly concentrated manures resulting from confinement-type livestock operations. The problem is most acute and the public is demanding a fast solution to this unsanitary and potentially dangerous health hazard.

Several of the States are engaged in programs of basic and applied research on the possible use of some of the various forms of electrical and physical energies as a means for improvement of the potential capabilities in farm production.



Investigations in progress, many of which are cooperative with the Department, involved the evaluation of the use of radiofrequency energy for treatment of grains to destroy insect infestation and treatment of seeds to improve their germination characteristics; exploration of the feasibility of using ultrasonics and electric shock to control rats, mice and birds; studies of the possibilities for a major advancement in the technology of small particle depositions through the application of electrostatic, thermal or other inertial forces; and use of light sources of various wavelengths for attracting and collecting insects which infest many of our economic crops.

The agricultural experiment stations of many of the States have research underway whose major objectives involve the obtaining of information on the uses to be made of electrical energy to reduce labor, increase production and improve family living conditions. In the design of these studies provision has been made to develop and investigate new equipment and explore the possibilities for new uses for electricity on the farm and in the home.

Many of the projects are concerned with the varied problems of chore labor mechanization and an expansion of the use of electricity for ventilating, heating, lighting and cooling under the various production enterprises of today's farming operations. Development and testing of prototype specialized equipment for product collection, processing, packaging, and transport, as well as crop storage, loading and unloading devices, are a part of the overall program of investigations.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Dairy cattle engineering

1. Increasing efficiency of operations. At Davis, California, studies to determine the effectiveness of herringbone milking parlors in reducing the labor requirement in large-scale dairy enterprises were continued in cooperation with the State Agricultural Experiment Station. Time and travel studies have been made on milking operations in a total of 54 herringbone layouts. Results to date indicate: (1) herringbone milking parlors are just as satisfactory for milking large herds (1000 cows or more) as for small; (2) herds can be milked faster (on a man-minutes per cow basis) in a herringbone parlor, with the same effort, than in any other type of milking layout -- assuming proper arrangement, good routine, and good equipment in proper adjustment; (3) herringbone layouts cost no more, and in some cases less, (on a per milking stall basis) than other types of elevated stall layouts; (4) herringbone layouts have little or no advantage over other types of elevated stall layouts for herds of less than about 35 cows; and (5) some operators place the milking machine on the cow between her hind legs, instead of from either the right or left flank, and believe this suspends the machine from the udder at a more natural angle.



Preparation of a manuscript of facility layouts covering several years' study has proceeded to the final stage. Some of the principles covered have already been used as the basis for technical papers and other publications.

2. Bio-engineering studies. Basic fundamental studies on the relationships between environment and various dairy animal health and production factors were continued in the psychroenergetic laboratory, and related facilities, at Columbia, Missouri, in cooperation with the Missouri Station.

Data from studies on the acclimation ability of lactating dairy cows conducted in the two preceding years were further analyzed. Exposure of the 10 lactating Holstein cows to the 85°F test condition for nine weeks caused significant decreases (from the 65° levels preceding and following the 85° exposure) for responses associated with heat production. Feed consumption decreased by 10 to 25 per cent and milk production decreased by about 15 per cent. At the same time, responses associated with heat dissipation increased. Only the rectal temperature and pulse rate showed statistically significant trends toward normal levels during exposure to heat.

Studies were continued on the effects of cooling inspired air for lactating Holstein dairy cows. Marked responses to the two levels of cooled inspired-air (60°F and 50°F) were obtained from treatment cows during both test seasons. Responses included increased feed intake and milk production, with accompanying decreases in rectal temperature and respiration rate. Although the values of each parameter approached "normal", they failed to reach it, indicating only a suitable total environment would provide maximum production and heat relief. Calculated cooling capacity required, based on cooling 28 cfm/cow of 85°F ambient air to 60°F, was 0.23 ton/cow. Actual cooling used in the experimental system for the same conditions, which included heat gains through the head enclosures and air distribution system, was about 1/3 ton/cow. For total barn air conditioning using the same conditions, a calculated capacity of about 2/3 ton/cow would be required.

Maintenance energy of non-lactating cows was studied using resting energy metabolism data of ad libitum and control fed (by rumen fistula) Holstein cows at various environmental temperatures. Initial results indicate the energy required for maintenance was minimal at 95°F; however, the minimal requirement at a normal body temperature was between 65 and 85°F environmental temperature.

A study comparing an air-conditioned dairy barn (maintained at approximately 75°F) with dry lot conditions for housing lactating dairy cows was conducted during the summer of 1964. Production, feed consumption, and rectal temperature of two groups of 16 cows were compared. A switch-back design was used involving three week treatments and three reversals of conditions.

Results for this first test season showed highly significant (1% level) differences in rectal temperature and milk production, with the air-conditioned barn providing the more favorable environment in both cases. Data obtained on feed consumption were not suitable for statistical treatment; however, the hay consumption in the barn was consistently less than that in the dry lot.

Field work on farm methods for cooling dairy cattle at Tifton, Georgia, has been discontinued. At the present time, the five years data involving shade and no shade during the summer, shelter and no shelter during the winter, and shade and shade in combination with fans and water sprays are being prepared for publication.

3. Plan development. At Beltsville, Maryland, in cooperation with the Northeastern Regional Plan Exchange sub-committee on dairy housing, sketches for Plan No. 3524, loose housing barn for young stock were developed. Further development will be needed before release to the Cooperative Farm Building Plan Exchange. In cooperation with the Southern Regional Plan Exchange Committee, a calf barn, Plan No. 5970, that was originally developed at North Carolina was released. This design features individual open-front pens for the calves and an enclosed feed room. It is expandable in modules of 12 feet (3 pens). Pole-type construction is specified.

All these plans but the first are included in the Cooperative Farm Building Plan Exchange.

## B. Beef cattle engineering

1. Hot, arid climates. These investigations are conducted at Davis, California, and at the Imperial Valley Field Station (El Centro) of the University of California, in cooperation with the Departments of Animal Husbandry and Agricultural Engineering of the California Agriculture Experiment Station.

Studies on sloping floors for beef cattle, as an aid in manure removal and pen cleaning were continued at El Centro. A fourth concrete floor (12 x 14 ft) was constructed and stalls were added to each pen so that 4 animals per pen had the following treatment.

Flat floor	- no stalls
Flat floor	- with 4 stalls
Sloped floor	- no stalls (slope 1 in/ft)
Sloped floor	- with 4 stalls (slope 1 in/ft)

The animals were on test from June 17, 1964, to December 2. Activity checks were made and manure was weighed. The results have not been analyzed completely, but it appears the stalls did not accomplish much and the sloped floor does have possibilities for manure removal. This work will be continued in conjunction with slatted floors.



A 112-day study of the effect of shades on beef cattle performance was started in June, 1964, at Davis. Thirty-two steers were divided into four groups, two on concrete and two on dirt. One pen of each of these had access to a 12'x24' shade (10 ft high). The following are the feed trial results.

	<u>Concrete</u>		<u>Dirt</u>	
	<u>Shade</u>	<u>No Shade</u>	<u>Shade</u>	<u>No Shade</u>
Av. daily gain	3.18	3.26	3.09	3.06
Lb feed/lb gain	7.83	7.70	7.69	7.35
Av. initial wt., lb	669	671	655	608
Total gain, lb	356	365	346	343

The average maximum and minimum temperatures during the period were 88.3°F and 53.0°F. There were no significant differences in the results indicating that shades are apparently not needed in an area like Davis for beef cattle. This will be repeated on an expanded scale.

Plans for a 35'x65' prefabricated metal laboratory building for beef cattle housing studies at El Centro have been drawn and construction is expected to be completed some time during the fall of 1965. The building will contain a main laboratory room that will hold twelve animals, individually penned, and three working rooms - all provided with equipment for controlled cooling.

2. Hot, humid climate. Inactive during reporting period.

3. Plan development. At Beltsville, Maryland, in cooperation with the Western Regional Plan Exchange Committee, Plans 5962 and 5969 were developed for tilting tables for handling calves. The first is a heavy duty wooden, permanent structure to handle calves up to 600 pounds; the second is a portable pipe-framed table designed for calves not exceeding 450 pounds.

Plan No. 5974, for a beef cattle corral, shows arrangement and dimensions of pens and facilities, but does not give details of construction.

These plans are all included in the Cooperative Farm Building Plan Exchange.

#### C. Swine engineering

Swine environmental studies were continued at Davis, California, in cooperation with the Animal Husbandry and Agricultural Engineering Departments of the California Agricultural Experiment Station. These include humidity effects and moisture loss measurements in a controlled laboratory, and production field tests on sprinklers, swine exercise, and ultra-violet lights for swine. Tests were continued at Tifton, Georgia, in cooperation with the Georgia Experiment Station, on sprinkling and shades for swine. There is no longer a USDA Agricultural Engineer at the Tifton station, but cooperation is maintained by periodic visits of the Davis personnel to Tifton.



1. Effect of humidity on swine. Humidity-growth studies were continued in the controlled laboratory at Davis. The third test in the series (identical to the second) was completed, using pigs initially weighing about 61 lbs each. Three pigs were placed in each of the three chambers inside the large test chamber. The relative humidity of each house was held constant at 45, 70, or 90% RH, while the dry bulb temperature of each house was maintained at the optimum for the weight of the pigs. A summary of growth for tests II and III are shown below.

<u>Average Daily Gain (Lb/day)</u>	<u>45% RH</u>	<u>70% RH</u>	<u>95% RH</u>
Test II	1.56	1.48	1.41
Test III	1.57	1.42	1.56
 <u>Lb Feed/Lb Gain</u>			
Test II	3.71	3.67	4.16
Test III	3.49	3.67	3.06

There is apparently no effect of relative humidity, in the ranges studied, when the dry bulb temperature is near optimum. The next test will be with dry bulb temperatures 10°F above the optimum.

2. Pig sprinklers for hot, arid climates. Studies on sprinklers for pigs in a hot-dry climate, at Davis, to determine nozzle size and operational regime, were continued in 1964. Five groups of nine pigs each were involved in the 63-day trial that began on July 1. Average maximum temperature was 89.5°F, average minimum was 54°F, and the mean was 70.6°F. The pertinent results are shown below.

Nozzle size (Monarch)	4.6	4.6	6.4	6.4	Control
Time on	On 15 Min	Continuous	On 15 Min	Continuous	-
	Off 15 Min		Off 15 Min		
Water use (gal/hr/pen)	3.08	6.16	4.09	8.18	-
Av. daily gain, lb	1.54	1.53	1.46	1.44	1.34
Lb. feed/lb gain	4.85	4.48	4.92	4.58	4.53

There were no significant differences between treatments. In a similar test in 1961, the control group had a highly significant reduction in gain below the sprayed group. The average mean, maximum and minimum temperatures in 1961 were 77.1, 96.8, and 60.1.

3. Sprinkling for hot, humid climates. Studies were continued at Tifton, Georgia, to determine the value of shade and shade-plus-fogging for growing-finishing pigs and for gestating sows and gilts in a hot, humid climate. The same movable shades on skids and lots were used as previously.

In 1964, 40 pigs (avg. 49.1 lbs each) were held in small pasture lots (10 pigs per lot), two lots had shade plus fogging under the shade and two lots had shade only. Again, an analysis of variance indicated a highly significant difference (at the 0.01 level) in rate of gain, but not in feed efficiency, due to the treatment.

In 1964, 16 bred sows and 16 bred gilts were in temporary pasture lots (8 animals per lot), two lots had shade plus fogging under the shade and two lots had shade only. Due to the unusual cool weather during the latter part of the gestation period, no rectal temperature or respiration rates were recorded. Again, the average number of live pigs farrowed, birth weight of live pigs, number of pigs weaned and adjusted 56-day weight of pigs appeared to be equal for the two treatments. This is the third year's data, and it is estimated that approximately four years' data will be required for analysis.

4. Stand-up feeding. A fourth stand-up feeding test was conducted with the following treatments: (1) control pen using ground-level feed troughs; (2) early stand-up, using elevated troughs from start of test; and (3) late stand-up, using elevated troughs only after pigs reached about 130 lbs. The results, which contained no statistical differences, are as follows:

	<u>Control</u>	<u>Early Standup</u>	<u>Late Standup</u>
Av. daily gain, lbs	1.57	1.56	1.60
Loin area, sq. in.	3.90	3.93	4.18
% ham	19.70	19.90	18.10
% loin	15.10	14.50	15.20

A treadmill, designed for 6 pigs, was mounted below one of the stand-up feeders, so that the pigs walk on their hind legs while eating, and was put in operation on February 3, 1965. The pigs are fed twice daily with the treadmill running about 2.5 inches/second for two hours at each feeding. The purpose is to see what controlled exercise might do to relative weight percentage and quality of different parts of the carcass, and the effect on feed efficiency. This test is still in progress.

5. Moisture loss. Studies on swine moisture loss were continued in the laboratory at Davis. A method was developed for measuring separately the moisture lost by swine through the skin and as respiratory loss from lungs and mouth. This is accomplished by using an airtight rigid plastic box to contain the pigs individually. By means of a fitted mask, respired air is exhausted from the box. The moisture content of inlet and exhaust air from the tent is measured. The increase in moisture with the mask off represents total vaporized moisture. With the mask on, only skin moisture is measured.

Some preliminary results are shown below. The effects of dew point (vapor pressure) and dry bulb temperature on moisture loss of 220 lb pigs are as follows:

<u>Temperature, °F</u>	<u>Moisture loss, grams/min at 53°F dew point</u>		
	<u>Total</u>	<u>Skin</u>	<u>Lungs</u>
84	1.27	0.61	0.66
68	0.96	0.50	0.46

  

<u>Dew Point</u>	<u>Moisture loss, grams/min at 84°F dry bulb</u>		
	<u>Total</u>	<u>Skin</u>	<u>Lungs</u>
71	2.10	1.35	0.75
53	1.27	0.61	0.66

6. Ultra-violet light. At Davis, California, 6 pens of pigs, 5 pigs per pen, were exposed to ultra-violet light at 2537 angstroms, 24 hours per day, from December 14, 1964 to February 23, 1965. Data are not yet analyzed, but there were no apparent significant effects of the light on health, gain, or feed efficiency.

7. Plan development. Plan No. 5947, Portable Shade for Hogs, was developed for the Cooperative Farm Building Plan Exchange. It is based on the results of research done at Tifton, Georgia.

#### D. Poultry engineering

1. Calorimeter studies. Investigations to determine heat and moisture production of broilers in the respiration calorimeters at Beltsville, Maryland were continued.

Studies with Athens Randombred (ARB) broilers were completed. Test temperature levels ranged from 5 to 30°C to 5°C increments. An additional study at 5 and 20°C was conducted with a commercial broiler (CB) strain to assess the engineering data obtained from ARB studies. All studies took day-old chicks through 9-weeks of age.

All brooding was started with calorimeter air temperatures ranging from 85 to 94°F and humidity near 60%. The temperature was dropped from brooding to test levels at various rates to find a suitable temperature reducing regime for the commercial broilers.

The preliminary heat production data per unit live-weight were similar for both strains. The CB birds, however, consistently grew at a faster rate. By the end of 9 weeks, the CB group averaged 1/3 heavier than the ARB's. Hence, both heat and moisture emitted per bird will be more for the CB than for the ARB.



Both strains emitted maximum heat per unit live-weight at 10 to 15 days of age. In previous tests with the New Hampshire x Cornish birds, the heat peaked near 25 days of age. This is an inference that modern broilers may be able to control body temperature much earlier than those of 15 to 20 years ago, indicating an opportunity for revising brooding temperature and reducing brooding cost.

The ARB birds demonstrated almost identical growth rate at any given test temperature level. These broilers had good growth rate between 50°F (10°C) and 77°F (25°C).

At 86°F and below 60% relative humidity, chicks 1 to 3 days old huddled excessively. Humidity of 75 to 80% at 86°F was apparently too comfortable as it appeared to decrease feeding activity.

The fresh fecal moisture content of both strains was within 80 to 83% on a wet basis. The higher value persisted at air temperatures above 60°F.

2. Southeast Poultry Research Laboratory. Engineering activity at this recently completed facility is largely still concerned with development, procurement, and installation of test equipment and instrumentation for the engineering portions of contemplated multi-discipline research programs.

During the report year, two prototype environmental cabinets, with control panels, were completed and partially performance-tested. A third control panel was almost completed. A third cabinet (of somewhat different design from the two previously constructed) is approaching completion. Shakedown and performance tests were made on the two completed cabinets. Test report is not yet available.

Each cabinet has separate temperature program control for cabinet top, bottom, and each sidewall, and for air temperature and air dewpoint within the cabinet. Controller response is rapid. Panel temperature change from maximum to minimum can be obtained in a couple of minutes or less. Humidity control has not been as good as hoped for, but this is expected to improve with further modification of the control system.

Four insulated rooms in environmental building number 8 were completed and are ready for installation of environmental control equipment upon delivery.

3. Field observations on relation of housing to disease in broilers in the South Central States. Work in Mississippi, in cooperation with AH and the Mississippi Station, has continued.

Insulation of the ceiling and the end walls of broiler houses in this area results in a reduction in fuel used for brooding, a very slight decrease of feed required to give a pound of gain in the winter time, and less likeli-

hood or death from heat prostration in the summer time when compared to houses having no insulation. Techniques for proper management of insulated houses need to be refined and taught to caretakers to obtain maximum benefits from insulation.

Except for the greater mortality experienced in extremely hot weather for a house with the ridge oriented in a N-S direction, no significant differences have been found in the production (weight, feed conversion, and condemnations) between houses with a ridge oriented N-S and those with a ridge E-W.

Studies comparing the performance of the new gas infrared brooder with the old standard canopy brooder indicated little difference in production results, although the infrared brooders consumed more fuel.

Studies to determine the adaptability of materials for broiler house curtain walls and their effects on environmental conditions inside the house have shown little advantage in production results for any of the materials studied.

Height of sidewall studies showed little production difference between broiler houses having wall heights of 6 1/2, 7, and 7 1/2 feet.

4. South Central Poultry Research Laboratory. Engineering research to determine by experimental procedures the effects of construction, equipment and management of poultry housing structures on broiler diseases and condemnations is being initiated at this newly constructed facility in cooperation with AH and the Mississippi Station.

Initial work will be conducted in four research poultry houses, each having four pens, on the laboratory farm and will be concerned principally with the influences of insulation and brooding equipment on selected environmental factors associated with the economics of poultry production.

5. Influence of turkey housing environment on disease. Work at St. Paul, Minnesota, in cooperation with the Minnesota Station, has continued. Turkey eggs have been dipped in chemicals in attempt to eliminate mycoplasma (PPLO) infections. The poults from these eggs have been grown in two types of housing and under varying environmental conditions. Although the egg dipping has not resulted in the complete elimination of all mycoplasma infections, it has resulted in a reduction of the airsacculitis caused by these micro-organisms. Further attempts to completely eliminate these micro-organisms will be made by combining the egg dipping with a medication program during the growing period.

During the winter months it was necessary to furnish supplemental heat to all test flocks. Attempts to lower pen temperatures below 50°F resulted in piling. This, along with the necessity of ventilating adequately to keep the ammonia at safe levels (below 20 ppm), appears to rule out minimum type housing for turkey broiler flocks.



The substitution of very small amounts of litter, added frequently, for thick layers of litter materials seems to have some advantages. The droppings do not become buried in the litter where the ventilating air cannot dry them.

Air sampling has revealed a very large population of micro-organisms per cubic foot of air. The bacteria counts reveal a uniformly high count (40,000 per ft<sup>3</sup>) throughout the growing period (8 - 14 weeks). Attempts to control these through the use of aerosol disinfectants will be made.

6. Plan development. The Arkansas Extension Service, the Cooperative Farm Building Plan Exchange at Beltsville, and the poultry sub-committee of the Southern Regional Plan Exchange, cooperatively developed a pole-type broiler house, 40 feet wide with length variable in modules of 16 feet. It features insulated hinged wall panels which may be opened for ventilation. The Plan Number is 5972, Broiler House, 3 sheets.

E. Livestock shades and shelters. Reported under B-1 and C-3.

F. Sky radiosity studies

Sky radiosity studies were continued at Davis, California (dry climate, clear sky) and at Columbia, Missouri (humid climate, overcast sky), in cooperation with the respective Experiment Stations. The quantity, quality, and variation of various parts of the sky are being studied in respect to the effect they may have on design and orientation of farm buildings and structures.

At Davis, California, two directional thermopile-type radiometers were used to measure the down coming diffuse sky radiation (wavelengths less than 3.5 microns) and atmospheric radiation (wavelengths greater than 3.5 microns) from various areas of the sky hemisphere. One radiometer responded only to diffuse sky radiation through a quartz filter, while the other one, covered with 2-mil polyethylene film for wind protection, provided a measure of total radiation. Atmospheric radiation was obtained as the difference between the two observations. Equations were developed for using the two-radiometer system to measure sky radiance. A field method of determining the absorptance of the polyethylene shield was developed.

Measurements were made at sea level (El Centro), 51 ft (Davis), 4,147 ft (Bishop), 9,200 ft (White Mountain), and 10,500 ft (White Mountain) during August and December of 1964. Some additional measurements were made in October of 1964. A set of measurements at any location was made once each hour from 9 AM to 3 PM inclusive. Total and diffuse sky radiation were measured at the zenith and at 45° starting from true north on azimuth circles of 15, 30, 45, 60, and 75 degrees above the horizon. Additional observations included irradiation on a horizontal surface and at normal incidence to the sun.



The spatial distributions of both downcoming diffuse sky and atmospheric radiation were found to be non-isotropic. The maximum flux density of diffuse sky radiation was often more than three times greater than the minimum that was observed from sky areas between sun azimuths of 120 and 240 degrees, and at elevation angles of 80 to 100 degrees from the sun's beam. Maximum amounts of both diffuse sky and atmospheric radiation were observed near the sun. The maximum flux density of atmospheric radiation was from 30 to 40 per cent greater than the minimum that was observed from sky areas between sun azimuths of 90 and 270 degrees, and near the zenith.

A reduction was noted in the amounts of both diffuse sky and atmospheric radiation as the site elevation above sea level increased. The diffuse sky radiation decreased at a faster rate, and the atmospheric radiation decreased at a slower rate, from sea level to 4,000 ft than from 4,000 to 10,500 ft.

Atmospheric radiation ranged from 73 to 91 per cent of total radiation; the averages for all data were 83 per cent atmospheric radiation and 17 per cent diffuse sky radiation.

Sky maps have been developed that show lines of equal flux density over the sky hemisphere as an aid in visualizing the spatial distribution of downcoming diffuse sky and atmospheric radiation. These indicate the magnitudes of the anisotropic distributions.

At Columbia, Missouri, after necessary calibration of the radiometer and recording potentiometers for this project, a series of four measurements were made. These included clear, cloudy, and partly cloudy days. Although the data have not yet been analyzed, preliminary examination has revealed some difficulties in the technique of measuring short-wave radiant fluxes from the sky. After further analysis, modifications in the method of obtaining short-wave radiation measurements may be developed.

#### G. Reducing pesticide residues in animal products

Development and testing of self-treating sprayers for control of biting flies on cattle was continued in cooperation with ENT at Kerrville, Texas. Field studies with 3 different automatic sprayers were conducted. Automatic sprayers pressurized with small electric air-compressors were simpler, more rugged, and more reliable than those with liquid pumps tested in 1963. Self-contained automatic sprayers, using pre-compressed oxygen or nitrogen for pressure and storage batteries for automation, controlled horn flies on range cattle but did not utilize the pre-compressed gases efficiently. Automatically controlled foggers that applied only 2 to 4 ml of material to each animal were subjected to preliminary testing and were partially successful.

A 1-ml "spot-treatment" for the control of horn flies on cattle was tested in the laboratory. The small volume of spray was applied daily to an area of about 1 square-foot on the withers of experimental animals, with concentrations of ronnel and malathion ranging from 1 to 10%. The spot-treatments were practically as effective as 50 ml sprays, applied daily, when similar amounts of active ingredient were used. The spot-treatments have the advantage of requiring simpler and cheaper sprayers than other methods, and automated systems may be constructed more easily. By applying the insecticide to areas on the foreparts of dairy cattle, it may be possible to reduce the chances of direct contamination of milk. Field tests and residue tests are planned for next year.

A series of 6 spray distribution tests were conducted. Spray distributions were determined by collecting hair samples from selected locations on the bodies of treated cattle, and analyzing the samples for insecticide content, using a simplified procedure for detecting coumaphos (Co-Ral). The tests indicated that 8-nozzle automatic sprayer booms would provide spray distributions as uniform as those provided by power-sprayer applications of 1 gallon. Nozzle arrangement and wind appeared to be the most important factors affecting the automatic sprayers. The weight of hair coats on animals treated by power sprayers had significant effects on the amounts of retained insecticide. Cattle sprayed in winter may retain 3 to 4 times as much insecticide on the hair coats as those sprayed in the summer.

**I. Farmstead manure disposal.** Laboratory and field studies are continuing in Maryland, in cooperation with the Maryland Station. Work this year was concerned almost exclusively with the hydroponic possibilities of lagoon effluent clarification and salvage of plant nutrients in the form of an animal feed crop. A 3-week laboratory test with the grass *Panicum Agrostoides* showed marked reductions in dissolved solids (82%). Green cut yields were in the range of 70-80 tons/acre inch of effluent. Effluent from two different lagoons was used, one serving a milking parlor and bottling plant and the other a swine farm. Distilled water was added for makeup. It was observed that the grass grew well in the effluent from the dairy lagoon but appeared to be dying in that from the swine lagoon -- leading to a subsequent finding that effluents may need to be "conditioned" for the purpose. This was borne out in tests with other, more desirable, grasses such as orchard, fescue, rye and timothy.

Ash analysis of fresh green cut tops was in the range 1.7 - 2.3%. Tests on nutrient levels will be performed.



## I. farmstead planning

1. Farmstead model layout studies. Studies on the use of models for analyzing farmstead layout problems have been continued in cooperation with the Minnesota Experiment Station at St. Paul, Minnesota. These studies show that such models are very useful in making a three-dimensional presentation of possible farmstead layouts. The successful use of models to analyze farmstead design problems in this study would indicate that most operators who are planning either a new setup or an expansion of their present layout might profit by using a scale model as one of the steps in planning their final layout design. The procedure to be followed in using the model method of planning is as follows: first, determine the management system and space requirements necessary in the final plan; the next step is to make and set up the models, moving them to all possible design locations before selecting the layout which best satisfies the specifications set forth in the first step; and lastly, plan the final construction, or, if it is an expansion program, plan a construction program to meet the needs of the increasing herd size.

2. Chore time standards. Studies on time standards for performing farmstead work elements are continuing in cooperation with the Minnesota Station, at St. Paul, Minnesota. A new series of studies was started to determine design and use recommendations for slat floors, free-stalls, and associated feeding equipment for dairy cattle. These studies are applying previously developed work element time standards to management systems using inside resting facilities and outside feeding. Observations are also being made to determine manure handling problems encountered with slat floors under both cold and warm operating conditions, bedding requirements for free-stalls, and feeding equipment layout arrangements for most efficient use of free-stall and/or slat floor systems.

An exploratory trial operation showed that it takes several days to get animals accustomed to using the slat floor, free-stalls, and animal operated doors. Prolonged freezing weather caused difficulties with the slat floor operation and indicated need for additional study to determine if the advantages of handling manure as a liquid during the greater portion of the year would justify the difficulties encountered during shorter cold weather periods. The animals remained exceptionally clean throughout most of the trial and indicated that properly designed paved walkways should keep them out of the mud.

## J. Physical Methods for Fly Control

Investigations of physical methods for controlling flies around dairies were continued at Beltsville, Maryland, in cooperation with the Animal Husbandry and Entomology Research Divisions, ARS.



The effectiveness of electrocutor-grid screens placed in windows plus an indoor electrocutor trap with blacklight lamps for controlling fly populations was evaluated in two calf barns where large numbers of house flies and stable flies were present. Although many flies were killed by the grids, no substantial reduction of the fly population within the grid-screened barn could be measured unless all major openings were closed or screened. When the animals were allowed access to exercise lots through open doors, the fly populations in the test barn and the "check" rapidly equalized.

Outdoor behavior of face flies was studied, both on herds in the field and on a single animal confined in a cage with a known fly population. Only a small proportion, 10 to 15 percent, of the total face fly population actually annoys cattle at any given time. Voluminous records were taken on the visits of individual flies to the animal in the outdoor cage in an effort to determine "typical" visiting behavior patterns. Analysis of the data is not complete, but some characteristics were apparent: Female flies visit the animals much more frequently than males, but males do cause some annoyance. The number of flies present on cattle is most closely related to the activity of the animals, with the greatest annoyance when the cattle are resting quietly. Although flies visit the face most frequently, they do rest on other parts of the body in considerable numbers. An evident peak of activity of released colonized flies occurred early in the morning, but activity of wild flies appeared more evenly distributed throughout the day. All flies leave the cattle at dusk while natural light levels are still quite high.

All these characteristics indicate the advisability of further investigating control measures which affect face flies when they are off the cattle.

Additional studies of the nocturnal habits of face flies confirmed that they rest on the foliage of trees at night. Although the flies are readily attracted (about 80 percent) to "blacklight" ultraviolet in confined spaces, those found resting on foliage at night do not respond in this manner. Electrocutor-grid traps with blacklight lamps placed in trees attracted less than 1 percent of a released population in 48 hours. Investigations of the factors affecting this change in behavior may provide information useful in control.

Laboratory tests of the mating activity of face flies indicate that sterilized males compete effectively with normal males in mating. Also, females appear to mate only once if they are inseminated during their first mating. Examination of females observed attempting to remate, only 5 to 10 percent of all females, revealed that they had not received any sperm during their first mating. This characteristic indicates that use of sterilized males should be effective in preventing reproduction. A laboratory trial using a ratio of eight sterilized males to one normal male to one female resulted in a 94 percent reduction in pupae.

Additional tests of the responses of face flies to monochromatic light confirmed that blacklight ultraviolet is highly attractive under conditions of confinement and that wavelengths in the red and yellow spectral regions are unattractive. Refinements of the testing procedure and methods of data analysis are being made in an attempt to more clearly establish differences in attractiveness.

#### **K. Dried Egg, Salmonella Studies**

Experiments at Pullman, Washington on the effects of glow-discharge radiation on Salmonella were continued. To minimize the effects of temperature on the bacteria, the irradiation chamber was packed in dry ice during treatment. Temperature measurements made inside the chamber indicated that complete destruction of the organisms was obtained at temperatures less than 30° C. These results indicate that temperature alone was not the cause of destruction. Further studies will be conducted.

#### **L. Automatic Equipment and Controls**

##### **1. Cattle Feeding Equipment**

In Illinois, work cooperative with the University of Illinois Agricultural Engineering and Animal Science Departments has resulted in the development of the satisfactory two-position automatic silo unloader control for upright silo unloaders. The two-position control utilizes a silicon control rectifier which can modulate the speed of an AC-DC motor that powers the silo unloader winch. A current transformer in the silo unloader motor circuit supplies a DC signal to the gate circuit of the rectifier to modulate the speed of the winch motor. With a maximum lowering rate of approximately 4 inches per minute, a delivery accuracy of 89 percent or better can be expected. This control system is economical. It uses readily available commercial components and can result in more satisfactory operation of the silo unloader than with manual controls.

In Minnesota the performance characteristics of electric motors for the operation of silo unloaders is being determined in cooperation with the University of Minnesota Agricultural Engineering Department.

During periods of sustained low temperatures freezing of silage in upright silos occurs. Starting at the wall the freezing progresses toward the center. The width of the frozen band depends upon the prevailing temperatures, the diameter of the silo and the nature of the silage material and its moisture content. Although knives are added to the gathering augers of unloaders it is generally known that output rates are reduced during the winter months.



Daily delivery rates were determined for two unloaders for a period of two months. The results indicate reduced output rates up to 50 percent at temperatures below  $-10^{\circ}$  F. The reductions would not be as great in larger diameter silos with a greater heat mass and in which the frozen area represents a smaller part of the surface area.

A cooperative project with Washington State University Agricultural Engineering Department on the development of the automatic trench silo unloader has not been active during the past year because of the absence of professional personnel to direct the project.

## 2. Hog Feeding Equipment

The cooperative research with the University of Illinois Agricultural Engineering Department has resulted in the design and field testing of a successful medium-pressure pneumatic conveying system for use with small feed grinders. The use of the patents for this device has been licensed to several manufacturers by USDA. One manufacturer produced two prototypes during 1964 and plans to produce five prototypes during 1965. Assistance has been given to manufacturers in the design of commercial models. A redesigned mill and conveyor system was placed on test in March 1964 on an Illinois farm. In March 1965 it had ground over 1600 tons of feed in nearly 1200 hours of use. Only one weak point was shown in the conveyor during this period of operation. Abrasion of the auger injector resulted in wearing through the injector tube. Thicker metal in this area will overcome this problem.

## 3 Poultry Equipment

Equipment was designed and custom built to record automatically the time of egg lay for 1000 birds in individual cages. This is the first known automatic recording trap nesting unit built in the United States. This equipment will be used to obtain detailed records for computer analysis on individual birds at the Beltsville Research Center.

## M. Environmental Control

### 1. Equipment for Poultry Environmental Studies

At Beltsville, in cooperation with the Poultry Husbandry Research Branch, the third year of a 5-year genetic selection of laying stock responsive to less than 24-hour day cycle (18 hours) was completed. A comparable control house was completed and used for birds housed on a conventional 24-hour day. Preliminary results indicate approximately equal production per season from birds on the 18-hour day as those on the 24-hour day.

Conditions maintained are  $75^{\circ}$  F. during light cycle,  $65^{\circ}$  F. during dark cycle and less than 75 percent relative humidity.

In Georgia, environmental facilities and instrumentation were designed and assembled to determine the effect of air velocity on the heat tolerance of



young chickens as indicated by body temperatures, respiration rates, and heartbeat rates. The equipment provided air velocities up to 570 fpm and temperatures up to 112° F.

Five different air velocities were maintained in one test and six air velocities in each of four additional tests, while the ambient temperatures were increased to maximum values ranging from 92° to 112° F. and then reduced to values near the original 68° to 88° F.

Increased air velocities were accompanied by longer delays in the rise of body temperature and respiration rate and smaller maximum values of these factors when the birds were subjected to increasing ambient temperature up to 105° F. The effect of air velocity on heart rate under the test conditions was not clear.

#### B. Equipment for Swine Environmental Studies

In cooperation with the Virginia Agricultural Experiment Station the study on buildings and equipment design for efficient swine production, including the utilization of heat pumps, has been continued. A summer test with Specific Pathogen Free hogs was conducted utilizing steel-slotted flooring in half of the environmental temperature-controlled buildings and in half of the semi-open house. Two pens of 15 hogs each were grown on the slotted floor and two pens of 15 hogs each on concrete floor in each building. On the steel-slotted floors the hogs' feet became tender and sore; however, the animals stayed cleaner and the floors were completely self-cleaned. As in previous tests, the average daily gains and feed efficiency data were not significantly different for animals in the two housing facilities. No difference in carcass quality analysis has been noted for hogs grown under the various test conditions in six trials. Hams from last trial are being cured under standard Smithfield procedures. These will be taste-test evaluated.

It has been concluded, on the basis of the data from six trials, that under the prevailing climatic conditions optimum temperature controlled housing was ineffective as a means of increasing swine growth rate and/or feed efficiency.

#### C. Milk Cooling Equipment on Farms

Experiments are being carried on to determine the minimum requirements for cooling capacity and operating conditions to accomplish satisfactory storage of raw milk for every other day pickup. The trials so far completed have been made on a 600-gallon ice-bank-type commercial farm tank, filled to near capacity with four successive milkings from animals in a research dairy herd at the Agricultural Research Center, Beltsville, Maryland. The rate of cooling of the milk has been varied experimentally by imposing an intermittent operating cycle on the cooling water circulation pump. Periodic samplings of the mixed milk are analyzed for free fatty acids as an indication of hydrolytic rancidity, for microbial growth, and for flavor deterioration.

Results indicate that for a raw milk supply of high initial quality, a cooling rate sufficient to bring the milk to 50° F. in two hours after milking is completed may be sufficient to maintain its quality. When the cooling time is increased to about four hours, bacterial multiplication can be demonstrated, although neither hydrolytic rancidity nor flavor deterioration are detectable.

Similar experiments will be conducted with lower quality raw milk. About 0.5 professional man-year is required for this study.

## PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

### Dairy Cattle Engineering

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## II. NUTRITION, CONSUMER USE, AND UTILIZATION RESEARCH

### NUTRITION AND CONSUMER USE RESEARCH

Consumer and Food Economics Research Division, ARS  
Human Nutrition Research Division, ARS

Problem. The assortment and characteristics of foods available to consumers change constantly with the adoption of new practices of production, processing, and marketing. Changing constantly also, as nutrition science advances, is our understanding of the nutritional needs of man and the manner in which these needs can best be met by food. To help meet the Department's responsibility to advise consumers on the quantity and variety of foods that will assure maximum benefit and satisfaction, research must continue on the nutritional requirements of persons of all age groups, on the nutrient and other values of foods and on how to conserve or enhance these values in household and institutional preparation and processing. Periodic surveys of the kinds, amounts, and costs of foods consumed by different population groups and individuals also are essential to evaluate nutritional adequacy of diets and to give the guidance needed for effective programs of nutrition education. Information from such surveys provides assistance needed in market analyses for different commodities and in the development and evaluation of agricultural policies relating to food production, distribution, and consumer use.

### USDA AND COOPERATIVE PROGRAM

The Department has a continuing program of research concerned with (1) nutritive and other consumer values of raw and processed foods as measured by chemical or physical means and by biologic response; (2) effects of household practices upon the nutritive values and inherent qualities of foods, and the development of principles and improved procedures for household food preparation, care, and preservation; (3) surveys of kinds, amounts, and costs of foods consumed by different population groups and the nutritional appraisal of diets and food supplies; and (4) development of guidance materials for nutrition programs.

The research is carried out by two divisions of the Agricultural Research Service -- the Human Nutrition and the Consumer and Food Economics Research Divisions. Most of the work is done at Beltsville and Hyattsville, Maryland; some is done under cooperative or contract arrangements with State Experiment Stations, universities, medical schools, and industry. The total Federal scientific effort devoted to research in these areas is 77.5 man-years. It is estimated that approximately 21.2 man-years is concerned with studies related to animal products.



Human metabolic studies and the related exploratory and confirmatory studies with experimental animals and microorganisms concerned with defining human requirements for nutrients and foods are not reported on a commodity basis, though some of the work is applicable to this report. This basic nutrition research represents a total Federal effort of 30.2 professional man-years and is described in detail in the report of the Human Nutrition Research Division. Certain aspects of this research related to animal products are considered in this report.

#### PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

Nutrient value of foods. Research on the nutritive composition of food is often concentrated on locally produced commodities. With the increase of processed and prepared foods, much of the evaluation is related not only to genetic factors and feeding practices but to changes induced by processing and storage to arrive at a value which represents the dietary contribution of the product.

The chemical structure of fats and lipids in food stuffs and the changes involved in processing, holding, and final preparation are receiving special attention. Protein and amino acid content and alteration with heat processing remain active research areas. The importance of conjugates of protein and lipids especially as they are formed in food processing, is being investigated in relation to their nutritive characteristics. Research has been directed toward the vitamin content of food as related to inherent inhibitory and stimulatory factors.

The total program in this area includes 38 projects in 24 States and is comprised of approximately 27.3 professional man-years.

Properties related to quality and consumer use of food. Research on food preparation for consumer use may be accompanied by measures of quality from the raw state, through handling and processing for marketing, to final home and institutional service. Special measures characterize certain classes of products; e.g., vitamin assays, enzymatic activity, water binding capacity, and changes in structural tissues.

The major research in product development is in the production, processing, and storage of beef, pork, lamb, poultry and eggs. Variables under study which affect the initial products include feeding regimens, age and breed. Conditions of processing relate to freezing temperature and duration and temperature of storage, shelf life, and the effect of light. Also being investigated is the effect of age and breed of animal, post-mortem aging, marbling and connective tissue on meat tenderness.

Special research emphasis is on the physical and chemical alterations involved in home preparation of foods. This work is carried out with the objective of having foods of maximum quality and nutritional value for final consumption. These researches include heat penetration studies of meats of different kinds and of varying fat content, suitable methods for home freezing and storage of fresh and precooked foods, micro-wave preparation of meats and the chemical changes involved, and flavor characterization in frozen and stored products by means of vapor component identification.



Many of these same factors are being investigated for institutional preparation where the quantities involved impose special conditions. Heat penetration and internal temperatures of a variety of meats as related to eating quality and yield is a field of intensive study.

The State program in this area includes 55 projects in 27 States and involves approximately 50.5 professional man-years. This is a partial report of the State Experiment Station program in food science and includes the work undertaken and participated in by Departments of Home Economics. For research on food and fiber, see the reports of the Utilization Research and Development Divisions, and Clothing and Housing Division.

Food consumption and diet appraisal. The State program in food consumption and diet appraisal extends and complements the work of the Department to selected segments of the population or to smaller geographic areas. One continuing investigation in the North Central Region is planned to yield information regarding food purchase and consumption patterns of families with preschool children. This research will provide information of use to both consumer education and market interests.

A continuing consumer panel in a Southern metropolitan area is providing information on purchase patterns, including data on changes in form, amount, kind, expenditure, and nutritive value of foods purchased. Attempt is made to identify and quantify in a relative sense the factors effecting change.

Currently 16 States are contributing to the Experiment Station program in this area which totals 22.7 professional man-years.

## PROGRESS--USDA AND COOPERATIVE PROGRAMS

### A. Nutrient and Other Consumer-Use Values of Animal Products

1. Beef. Broiled rib steaks were scored rare when broiled to an internal temperature of 140° F., medium when broiled to 160° F., and well done when broiled to 180° F. On the other hand, eye of round steaks broiled to 140° F. were medium in doneness and those broiled to 160° to 180° F. were well done. Rib steaks from more marbled carcasses contained less moisture and myoglobin and were scored higher in tenderness, juiciness, flavor, and general acceptability than steaks from less marbled carcasses. These differences were not found in steaks from the eye of round. For the most part, scores by a small expert panel for tenderness, juiciness, flavor, and general acceptability decreased as steaks (rib or eye of round) were broiled to higher internal temperatures.

Research was initiated on changes in color and tenderness of beef steaks as influenced by different rates and extents of heating by broiling. Steaks from chuck, rib, loin, and round portions of beef carcasses were broiled at

temperatures of 250°, 350°, or 450° F. for different lengths of time. Changes in color, measured visually, instrumentally, and chemically, and in tenderness, measured instrumentally, will be associated with rate and extent of heating and with other factors, such as fat and moisture, that may influence rate of heat penetration.

Frozen, boneless beef roasts from mature animals, yearling, and calf, and frozen ground beef and veal have been distributed to schools participating in the National School Lunch Program. To provide information on appropriate preparation procedures and the palatability and yield of cooked meat, roasts were cooked from the frozen state by braising and by roasting and ground meat was thawed and oven-cooked in the form of patties. The results were used as a basis for recommendations to schools.

Research has been initiated under contract with the University of Wisconsin at Madison, on the kinds and amounts of fatty acids in triglyceride and phospholipid fractions from fat and lean tissues of cuts of beef from the shoulder, rib, flank, and round. Analyses will be made on both raw and cooked meat. Meat from the shoulder and flank will be cooked by a moist heat method and meat from the rib and round by a dry heat method. As a part of this research biopsies of the longissimus dorsi muscle of beef animals will be made at intervals corresponding to customary market ages. The lipid portion of the muscle tissue will be analyzed for fatty acid content. This part of the study was undertaken to provide information on the effect of age of the animal at slaughter upon the fatty acid content of beef as it is available to the consumer.

2. Beef and pork. The study of changes of the fatty acid composition of the fat of ground beef and of ground pork patties due to cooking has been completed in a research contract with the University of Tennessee at Knoxville. The fatty acid composition of total lipids, neutral fat, and phospholipids was determined. A manuscript presenting the findings will be prepared.

3. Pork. A study was carried out in cooperation with the Meat Inspection Division of the Consumer and Marketing Service on the effect upon eating quality of pork sausage of the addition of antioxidants during processing. The antioxidant used was a combination of butylated hydroxyanisole and butylated hydroxytoluene. The sausages were stored under various conditions representative of marketing and household practices. The links were evaluated at intervals over a period of 16 weeks storage at 0° F. Color and odor of the raw sausage and flavor of cooked sausage were evaluated for the freshly thawed links (24 hours at 45° F.) and after an additional four-day holding period at 45° F. The findings are being evaluated and a manuscript will be prepared.



4. Poultry. Research has been initiated under contract with the Food and Drug Research Laboratories, Inc., at Maspeth, New York, on possible changes in nutrient composition of eggs associated with the use of a pesticide, malathion, in management of the hens. The possible effects upon the eating quality of meat from the hens also will be evaluated. The nutrients to be studied in the eggs are the fatty acid composition of the egg lipids, vitamin A-carotene, cholesterol, and individual amino acids.

The first in a new series of consumer publications has been published--Home and Garden Bulletin 103 "Eggs in Family Meals: A Guide for Consumers." Another bulletin in preparation for the series will provide information on poultry. The new publication on eggs gives suggestions for buying and storing eggs, the principles of egg cookery, and a wide assortment of new recipes in which eggs are an essential ingredient. It also tells about selection and use of dried and frozen egg products. Each bulletin in the new series will bring together the research findings on phases of food preparation, including guides for buying food, nutritional information, up-to-date cooking methods, servings to be expected from certain amounts, and menu suggestions.

Studies of eating quality, yield, and heat penetration of defrosted turkeys stuffed and roasted to end points of 180°, 185°, and 190° F. in the thigh were completed. Increases in the end point temperature in the thigh increased cooking times and doneness and decreased juiciness. An end point of 185° F. in the inner thigh gave the best indication of optimum eating characteristics of the cooked meat. Use of hot stuffing in the colder turkeys decreased cooking times. However, this procedure extended the time the stuffing remained in the temperature zone where maximum growth of food poisoning bacteria could occur and, therefore, is not recommended as a safe practice.

Investigations were continued on the effect of cooking and end-point temperatures upon eating quality and yield of turkey roasts. Light meat turkey roasts and dark meat turkey roasts were roasted at 250°, 325°, and 400° F. to end points of 165°, 175°, 185°, and 195° F. The results have been evaluated and a manuscript is being prepared. Laboratory work also has been completed on the eating quality of both light and dark meat turkey roasts braised at oven temperatures of 250°, 300°, 350°, and 400° F. to end points of 165°, 175°, 185°, and 195° F. The data are being evaluated. Boneless turkey roasts weighing 9-1/2 to 11-1/2 pounds each and made with light and dark meat were cooked from the frozen state for comparison with turkey roasts cooked after thawing. Methods of cooking included roasting at 350° F. and braising at 350° or at 450° F. to a specified internal temperature of 165° or 170° F. under conditions prevailing in school food service units. Eliminating the thawing of frozen roasts before cooking would be desirable in some food service units because of the possible danger from food poisoning organisms. Large boneless turkey roasts were cooked

satisfactorily by any of the procedures used in this study. Frozen roasts without thawing could be roasted or braised within 4-1/3 hours and thawed roasts within 3-1/3 hours. Thawing roasts of this size required about 18 hours in a refrigerator.

Research under contract with Purdue University at Lafayette, Indiana on the microbial and quality characteristics of turkeys stuffed and roasted under different conditions was completed. A total of 230 frozen ready-to-cook tom turkeys weighing 12 to 24 pounds each were roasted at 200°, 325°, or 450° F. Dry and moist stuffings were used and turkeys were stuffed just before cooking or stuffed and refrigerated overnight. Based on results of this and other research in the HN Division, roasting time tables in the Department's consumer bulletins have been revised to reflect modern practices as well as production and processing methods currently in use. Total cooking times recommended for stuffed whole turkeys weighing from 12 to 24 pounds were reduced by 1/2 to 2 hours. The reduction in cooking time increased with the size of the turkey.

## B. Nutritional Evaluation of Animal Products

1. Heated and oxidized fats. The effect of oxidation upon the nutritive value of different dietary fats has been followed in long-term studies conducted under research contracts with Columbia University, New York City, and with Swift and Company, Chicago, Illinois. Mild oxidation of beef fat, chicken fat, olive oil, or cottonseed oil had little influence on the lipid composition of the tissues when fed to rats. These fats were oxidized at 140° F. by aeration for a period of 40 hours. Continuing studies will provide information for butter and lard. The results also suggested that the body is able to rearrange the constituent fatty acids as supplied by the dietary fat and, despite similarity in total fatty acid composition, each organ appears to fashion its own triglycerides. A paper reporting these findings was presented at the meeting of the American Oil Chemist's and is being prepared for publication.

Other studies evaluated the influence of more severe oxidation produced by heating hydrogenated vegetable oil, cottonseed oil, corn oil, and lard without aeration for 120 hours at 360° F. These conditions are somewhat more drastic than would be encountered with the mild treatment that fats usually receive in the home or under the conditions of commercial use, but are still comparable to some food preparation practices. The results obtained with the fats investigated to date indicate that any changes due to heat treatment were without harmful effects as determined by the physiological response to diets containing these fats. Animals fed the heated fats ate more than those fed unheated fats, with the greatest differences being observed in the intake of those fed lard or cottonseed oil. When fed heated or unheated hydrogenated vegetable oil, the animals lived somewhat longer than those fed cottonseed oil, corn oil, or lard. These results were reported at a meeting of the Federation of American Societies for Experimental Biology and a paper will be prepared for publication.



2. Dietary fat and insecticides. Research is being initiated that should aid in establishing whether the presence of the chlorinated hydrocarbons in food could influence metabolic response during growth and reproductive stress and periods of dietary restriction. Under contract with Swift and Company, Chicago, Illinois, investigations will be conducted to determine the effect of feeding diets containing selected types of heated and unheated fats, with and without added chlorinated hydrocarbon pesticides, on growth and reproductive performance of the rat through three generations. The heated fats will be prepared under conditions similar to those used for a previous study (B-1 above). Selected tissues will be analyzed for pesticide residues to determine the extent to which these pesticides may accumulate in the tissues without harmful effects. The pesticides will be added in amounts that will not exceed currently accepted tolerance levels and will be in the proportions found in composite diets recently analyzed by Food and Drug Administration.

At Beltsville, the effect of the same mixture of pesticides on body chemistry and tissue structure will be investigated to determine the extent to which these pesticides may be stored in the adipose tissue of the obese rat, and to assess the physiological response when these pesticides are liberated from adipose tissue during a period of active weight loss. The diets used will contain adequate amounts of essential nutrients and have been selected on the basis of ready acceptability, resulting in heavy and obese rats when fed ad libitum. One diet is high in fat; a second contains a relatively high concentration of sucrose and moderate amounts of fat; in the third diet the carbohydrate is chiefly starch and the level of fat is low.

3. Dietary fat and serum proteins. Fatty acids are known to complex with certain protein components in blood as a means of lipid transport. Research to determine the possible effect of the kind and level of dietary fat on the relative proportion of various serum protein components has been carried out in order to obtain basic information on the response to dietary fat and to aid in our understanding of the role of the blood proteins in the transport of fat. Fats used in the study were corn oil, safflower oil, hydrogenated vegetable oil, lard, and butter. Serum proteins were analyzed electrophoretically using moving boundary electrophoresis. Both age of animals and kind of dietary fat were found to influence the concentration of certain of the protein components in the blood of rats. A component moving more rapidly than albumin and containing both fat and protein, was found to be particularly susceptible to the kind and level of fat in the diet as well as to age of the animal. Differences due to kind of fat were not related to any specific characteristic of the fat. The functions of these fat-protein complexes remain to be elucidated.

4. PL 480 studies of diet and fat metabolism. Among factors known to affect fat metabolism are genetics, age, and other physiological and environmental including numerous dietary factors. In one PL 480 project in India, the effect of diet on hormone regulation of body synthesis and mobilization of

fat is being studied with rats. One group of animals is receiving a fat-free laboratory diet, and others are receiving protein and fat combinations characteristic of three population diets in North, South, and Central India. For comparison other rats are maintained on regular stock ration.

In normal animals, the North India combination of 20 percent animal protein (casein) plus 20 percent butterfat caused significant rise in serum cholesterol. The South India combination of legumes (vegetable protein about 10 percent) and 10 percent coconut oil caused high initial serum cholesterol which in 30 days fell to levels below those on the stock ration. The South India combination of another vegetable protein and 10 percent sesame oil gave the lowest serum lipid levels of all. Rats on fat-free diets of 18 percent casein and 64 percent cornstarch and 12 percent sugar had serum cholesterol significantly above animals on the stock ration or on any of the population type of diets. Maximum synthesis of body fat occurred also on the fat-free diet. Irrespective of the diet used, removal of the thyroid gland led to higher serum cholesterol but it was most exaggerated on the North India combination. Administration of thyroid hormone tended to reverse the effect on all diets. Removal of adrenal glands depressed lipid metabolism but the effect was only partially corrected by corticosteroids, one hormone produced by the adrenals.

5. Amino acid patterns in food proteins. Statistical analyses of nitrogen-balance data for 24 young women fed diets containing the FAO pattern of essential amino acids and the patterns in nonfat milk solids, whole egg, oatmeal, and peanut butter were interpreted as indicating (a) that young women, when fed amino acids as in the FAO pattern, required a minimum of .22 gm tryptophan and other essential amino acids as in this pattern to maintain nitrogen balance, (b) that the nutritional value of a protein depends in part upon factors other than the amount of the limiting amino acid, and (c) that one possible factor may have been the larger amount of nitrogen from essential amino acids in the food patterns than in the FAO pattern. The data were obtained in contract research with the University of California at Los Angeles, Oklahoma State University at Stillwater, and the University of Wisconsin at Madison. A manuscript presenting an evaluation of the combined data has been accepted for publication in the American Journal of Clinical Nutrition.

6. Milk products. Laboratory investigations to determine the nutritional value of various components of milk have been completed and will provide data on the response of rats to diets containing dried skim milk and butter oil and to milk protein with various combinations of fat, as butter oil or corn oil, and carbohydrate, as lactose or cornstarch. The results are being evaluated and will be prepared for publication.



### C. Tables of Food Composition

1. Beef--proximate composition, from carcass to cooked meat. A research report describing the procedure developed for obtaining the data on beef in Handbook No. 8 was completed and will be published as Home Economics Research Report No. 31. It provides data on the physical and chemical (proximate) composition for (1) beef carcasses of six grades--prime, choice, good, standard, commercial, and utility; (2) retail cuts made from carcasses of the three most important grades with an ordinary amount of trimming; (3) retail cuts of the three grades completely trimmed of all separable fatty tissue; and (4) cooked meat prepared from these retail cuts. The details of the procedures for deriving the data on the composition of beef at each step with appropriate relationships among grades and among cuts within each grade are given in the report.

2. B-vitamins and trace elements in foods. Compilation of data is well underway for the following B-vitamins--pantothenic acid, vitamin B<sub>6</sub> and vitamin B<sub>12</sub>, and has been initiated for several trace elements. Data for these nutrients were not given in the 1963 revision of Agriculture Handbook No. 8 "Composition of Foods...raw, processed, prepared." Special attention is being given to compiling data on cobalt, copper, manganese, molybdenum, selenium, and zinc.

3. Nutritive values of retail and household units of food. A table is being developed which will give nutritive values for many of the foods in Handbook No. 8 in terms of market units of the items as usually purchased and in terms of household measures of prepared foods. The data needed to prepare this supplement for the 1963 edition of the Handbook are being obtained through consultation with representatives of industry and of Consumer and Marketing Service as well as through observation of products offered for sale.

4. Special services. Research findings compiled from the world's literature on the nutritive value of foods continue to be in constant demand as background material for dealing with a wide variety of problems. For example, technical assistance was given in the formulation of a statement on nitrogen conversion factors for the Protein Committee of the Food and Agriculture Organization of the United Nations and in the development of policy and guidance for the Food for Peace Program. Information on the composition of specific foods or groups of foods was also given to research teams conducting dietary surveys, to welfare workers and to such agencies as the Federal Trade Commission, the Food and Drug Administration, and the National Institutes of Health. Within USDA, information was provided for revising several tables in Statistical Bulletin No. 362, Conversion Factors and Weights and Measures for Agricultural Commodities and Their Products, issued in June 1965.

### D. Food Consumption and Diet Appraisal

1. 1965 nationwide survey. A nationwide survey designed to provide information on the food consumption and dietary levels of people in the United States is now underway. Data on the kinds and quantities of food used during

one week were collected under contract from more than 7,500 representative U.S. households between April 7 and July 3. Similar data will be obtained from 2,500 households each in the summer and fall of 1965 and the winter of 1966. Information on the food eaten both at home and away from home during one day was provided by 13,000 individuals who were members of the families providing information on household food consumption during the spring of 1965. The study was designed to provide data for the four Census regions for farm, rural nonfarm, and urban populations for the year as a whole and for the four seasons.

Detailed tabulation plans have been drawn up that will provide for a series of volumes on the household data obtained in the spring of 1965 similar to those published for the 1955 survey. Other tabulation plans will provide information on (1) the 12-month period April 1965-March 1966 and for the four seasons, (2) the relationship between the money value of household food and its dietary adequacy, and (3) the intake of both food and nutrients of individuals by age and sex.

## 2. Effects of food distribution programs on diets of needy families.

Analyses of data from studies in Detroit, Michigan, and Fayette County, Pennsylvania, conducted to obtain information on the effectiveness of the Food Donation and Food Stamp Programs showed the following. (1) Many of the families participating in the Food Donation Program failed to participate in the Food Stamp Program when it replaced the Donation Program. The families who did participate in the Food Stamp Program were usually those of younger homemakers with more formal education, more young children, and lower incomes for family size. (2) Calcium and ascorbic acid were the nutrients which increased most when the Food Stamp Program replaced the Food Donation Program. They were also the nutrients in which diets were most limited (according to the National Research Council allowances) under both the Food Stamp Program and the Food Donation Program. (3) The overall quality of diets was better under the Food Stamp Program than under the Food Donation Program. (4) Under the Food Stamp Program participants received coupons which would purchase food worth considerably more in money than the foods received under the Food Donation Program--approximately 135 percent more in Detroit and 65 percent more in rural Fayette County. They were also able to make their own selections.

A study of families participating in the Food Donation Program in Baltimore showed that little or no dietary improvement occurred when the number of donated foods was increased from five to eight. Families cut back on their food purchases, apparently using the funds thus made available for other needs.

## 3. Food consumption of the rural population in Spain (PL 480 Research).

A 1964 survey of the food consumption of the rural population in Spain, conducted by the Spanish Ministry of Commerce under the cooperative sponsorship of the Economic Research Service and the Agricultural Research Service,



using PL 480 funds, showed the percentage of income spent for food was much higher than in the U.S. However, the nutritive content of the diet was considerably lower than that of rural families of the U.S. For example, the average amount of calcium in the diet was about 0.5 g. per person per day compared to 1.2 g. in the diets of rural families in the U.S. in 1955. The percentage of calories from fat was 31 percent compared to 41 percent in the diets of rural families in the U.S. Although a wide variety of foods was used, a large share of the diet was supplied by bread, potatoes, dried beans, milk, olive oil, and wine. Tabulation of data from a second survey conducted in the early months of 1965 is in progress.

4. Household practices in home freezer management. Field work has been completed for the study of management practices of owners of home freezers. The information was obtained from 200 urban families in Fort Wayne, Indiana, and 200 farm families in the surrounding area. Plans made for tabulating the data will make it possible to describe for groups of families of homogeneous economic characteristics, the types of freezers owned, values attributed to freezer ownership, length of time foods are stored in freezers, and other freezer management practices.

5. Nutritive value of national food supply. The revision of estimates of the food energy, protein, fat, carbohydrate, and selected vitamin and mineral content of the per capita food supply from 1909 to the present has been completed. The revised figures incorporate newest estimates of per capita food consumption developed by the Economic Research Service, revised food composition data from Agriculture Handbook No. 8, and new information on the nutrients added to foods by enrichment and fortification. The revised estimates and tables showing the contribution of major food groups to the total supply of each nutrient for selected years were published in Chapter 5 of Statistical Bulletin No. 364, "U.S. Food Consumption--Sources of Data and Trends," Economics Research Service, June 1965.

The estimates for nutrients together with the per capita food quantities on which they are based are extremely useful in studying dietary trends. For example, the total protein content of the per capita food supply has remained fairly stable in the last 50 years, but the share from animal products has increased during this period from about half to more than two-thirds of the total. Contributing to this shift are increasing consumption of beef and poultry and decreasing consumption of grain products.

6. Support for food and nutrition programs. The compiling and interpreting of research-based information on nutrition for application to problems of food selection and food use is continuing. The information so developed serves as a basis for assistance to many groups both within and outside the Department. For example, technical advice and guidance were given to the School Lunch Division, Consumer and Marketing Service, in revision of two

publications designed to help improve the nutritional quality of school lunches. Also assistance was given to the Office of Economic Opportunity in the preparation of "Nutrition Guidelines" for the Project Head Start Centers Feeding Program.

Publication of Nutrition Program News and participation in the Interagency Committee on Nutrition Education, for which CFE furnishes the secretariat, are continuing as a means of coordinating and strengthening nutrition programs in general. A noteworthy accomplishment was the development by the Committee of four basic nutrition concepts to be used as guidelines for program planning and curricular development in nutrition education.

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**MEATS - PROCESSING AND PRODUCTS**  
**Eastern Utilization Research and Development Division, ARS**

Problem. Livestock production is our greatest single source of farm income. For the past several years over 30 percent of cash receipts from farming were derived from livestock sales. Likewise, the major portion of our land is used to grow livestock feed and forage. Hence, any research which succeeds in stimulating an increase in the consumption of meat and livestock products will have a profound effect on agriculture as a whole. For example, it is estimated that a one percent increase in meat consumption would require an increase in feed equivalent to 80 million bushels of corn.

The processing of meat and meat products also has an important effect on rural industry and rural employment. About half of our meat supply is derived from packing plants in rural areas. Many of these are small (the state of Pennsylvania alone has over 2,000 registered slaughterers) and cannot hope to maintain their own research facilities. They employ local labor, and their products are transported and sold by local truckers and business men. Thus, increases in meat consumption and improvements in meat technology will contribute to increased rural prosperity.

Increases in livestock consumption may be achieved through development of new or improved meat products, or through improved meat processing technology which results in lower costs. In addition, increases in the value of hides, animal fats, and renderers' proteins will benefit the livestock industry by providing additional revenues which could permit reduction in meat prices (thus stimulating consumption) or which could flow back through the marketing channels in whole or in part to livestock growers and feed producers. For example, it is estimated that loss of the market for hides would cause an increase of meat prices that would result in a decrease of 2 percent in meat consumption. Such a decrease would eliminate a market for feed equivalent to 160 million bushels of corn. Conversely, an increase in hide values would operate in the opposite direction and would result in greater income to the livestock industry and in increased utilization of feed grains.

Increased livestock consumption requires both basic and applied research. Applied research is the forerunner of commercial practice and is an indispensable element in successful development. But applied research depends on new knowledge which must be developed in fundamental studies. Our supply of fundamental knowledge must be maintained and expanded if applied research is to be effective and fruitful. This need for basic research has been pointed out by the Animal and Animal Products Research and Marketing Committee, by the Utilization Research Advisory Committee, by the National Agricultural Research Advisory Committee, and by other responsible meat industry groups.

For the reasons given above, research which succeeds in increasing meat consumption can have a powerful effect on American agriculture. The potential effect may be assessed from the facts that meat has a high elasticity demand (a 1 percent drop in retail prices will result in a 0.7 percent increase in



consumption); the production of one pound of livestock requires the equivalent of 7 to 8 pounds of feed grains; and the present United States consumption of meat (174 lb./person in 1964) is still below that of Australia (234 lb.), New Zealand (222 lb.), or Uruguay (234 lb.). Economists predict that the 1965 United States consumption will be lower than in 1964.

Attaining increased meat consumption and providing new technological information for small processors will require a vigorous and balanced research program. There is need for more applied research on processing and preservation, including expanded studies on increasing the efficiency of sausage production and on new dried or semi-dried and ready-to-eat products. Of even greater importance is the need for more basic research on the physical, chemical, and microbiological properties of meat to provide a fund of knowledge for future technological improvements.

#### USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving chemists, biochemists, microbiologists, and food technologists engaged in both basic studies and in the application of known principles to the solution of problems in the processing of meat and meat products. The Department's research facilities are located at Beltsville, Maryland, and at Wyndmoor, Pennsylvania.

The Federal scientific effort devoted to research in this area totals 31.4 professional man-years including 6.8 p.m.-y. of contract and grant research. This effort is applied as follows:

(a) Research on microbiology of meat and meat products involves 7.6 p.m.-y. at Beltsville, Maryland. In addition, contract research at Iowa State University (1.6 p.m.-y.) seeks to develop new cured products of distinctive flavor through a study of the fungi associated with cured meat.

(b) Product stability studies involve 4.7 p.m.-y. at Beltsville, Maryland. A grant at Florida State University (1.0 p.m.-y.) is devoted to a study of the relationship between heme pigments and oxidative rancidity in cooked and frozen meats. Another grant at Rutgers University (0.6 p.m.-y.) has been awarded for the study of the non-carbonyl compounds associated with rancid meat. A research contract at the University of Missouri (1.0 p.m.-y.) is devoted to the study of flavor stability and the time-temperature-tolerance of precooked, frozen meat products.

(c) Study of meat composition and quality involves 6.6 p.m.-y. at Wyndmoor, Pennsylvania. A research contract at Louisiana State University (1.4 p.m.-y.) is concerned with the modification of muscle connective tissue constituents and their relationship to tenderness.

(d) Research on meat flavor and smoke composition occupies 5.7 p.m.-y. at Wyndmoor, Pennsylvania. In addition, there is a research contract devoted to the design of new smoked meat products at Michigan State University (1.2 p.m.-y.)

## PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

For many years State stations have conducted both basic and applied research on problems related to meat utilization. Program scope extends from study of the effects of pre-slaughter treatments on meat quality to development of new or improved meat products.

Pre-slaughter factors such as the effect of breed, diet, stress, environmental conditions and hormonal supplementation are evaluated in terms of the ultimate relationships to quality, color, water binding capacity, structure and composition of meat. Physical and biochemical characteristics of meat are also related to flavor and acceptability of the meat. Such factors as marbling and maturity have long been implicated as factors affecting the utilization of meat. For example, recent results indicate that wide extremes in carcass maturity influence the eating quality of beef a great deal more than wide extremes in marbling.

Among the basic studies related to meat utilization is the continuing program designed to discover the biological mechanism of muscle contraction and to elucidate its relationship to meat tenderness. Study of post-mortem glycolysis in portions of the same pork muscle, which differ markedly in red fiber content provides a unique opportunity to estimate comparative differences in the rate and extent of glycolysis. Effects of post-mortem temperature on pH decline, glycogen depletion, lactic acid accumulation, muscle color and expressible juice are being measured. Other work seeks information on the association of various physiological factors to the post-mortem properties of musculature.

Since the major hydrolytic enzymes of animal tissues are organized within sub-cellular organelles (lysosomes), the relationship between the chemical composition, structure and function of lysosomes is being determined. Lysosomal enzymes are important in many processes which occur in meats including those of post-mortem autolysis, aging and tenderization and hydrolytic production of flavor constituents. Study of the components of lysosomes has revealed that the lysosomal enzymes include cathepsins, nucleases and glycosidases.

Considerable research effort is devoted to isolation and characterization of meat proteins. Proteolytic changes occur in beef muscle during aging. Muscle proteins are being extracted, fractionated, and their properties used to follow changes occurring during aging. Sarcoplasmic proteins have been fractionated into 18 components. Absorption spectra indicate that these fractions contain several enzymes directly involved in muscle metabolism. Since the major portion of the catheptic activity of beef muscle occurs at pH 4, initial effort will be aimed at purification and characterization of this enzyme. There is need for a rapid, quantitative assay technique for proteolytic enzymes in meat products and further work will be directed along these lines.

The chemical nature of meat pigments continues to be studied. The color stability of freeze-dried meats and problems of pigment fading in cured meats



are of interest. Reflectance spectrophotometry methods are being developed for use in the analysis of pigments at the surface of fresh meat and should help to better understand meat discoloration due to oxidation of myoglobin. The appearance of prepackaged meat is important to consumers who often assume color of meat to be an indication of fresh quality. Research seeks the cause(s) of meat discoloration and means for control of meat color.

As is the case with most foods, investigations of the components of meat flavor are in progress. Workers seek to determine the role of fatty acids, volatiles and amino acids in flavor. Histological studies evaluate structural changes and relate these to eating quality.

Microbiological research is concerned with the microbial aspects of cooking, packaging, storage, curing, fermentation and aging of fresh, prepackaged and processed meat items. Radiation resistance of certain bacteria is being investigated.

An extensive program of work is directed to development of new products and processes. The problems of processing are approached through study of reactions which occur during curing, smoking, aging, freezing, freeze-drying and prefabrication. Product stability studies are also in progress.

A regional project, WM-33, covers a portion of the meats research reported.

The total research effort on meat is carried out at 41 stations and involves approximately 60.6 p.m.-y.

#### PROGRESS -- USDA AND COOPERATIVE PROGRAMS

##### A. Microbiology of Meat and Meat Products.

The mechanism by which some microorganisms can grow at low temperatures, the so-called "psychrophiles," is being investigated with a strain of Escherichia coli through radioactive tracer studies of the interrelationships of cell yield, temperature and substrate utilization. Present data indicate that more energy is required to maintain or produce a given level of cells at low temperatures than at higher temperatures. Additional studies in this area should indicate the metabolic pathways involved.

Continuing studies on microbial activity on fats have shown that the lipase from a psychrophile, Pseudomonas fragi, is truly extracellular. Disc electrophoresis has shown two bands of lipolytic activity instead of the single activity previously noted, and purification by density gradients on Sephadex columns has eliminated a proteinase contaminant. Studies on specificity with this enzyme should now be more reliable. The lipase previously isolated from Geotrichum candidum has been shown to be stereo-specific for the cis-form of oleic acid. This is not true with pancreatic, milk, or wheat germ lipases. This specificity may prove useful in the study of the triglyceride structure of natural fats and oils.

Investigation of the role of microorganisms in non-hydrolytic rancidity in fats has shown that a number of microorganisms actually utilize carbonyls and peroxides. It also has been found that the activity of some microbial lipases is severely limited by certain unsaturated fatty acids.

Food poisoning caused by Clostridium perfringens occurs rather frequently in Europe and sporadically in the United States. Although it is usually associated with meat and meat products, it is rarely related to cured meats. Studies of the past year on the effect of curing salts and smoking on the growth and survival of this sporeformer in cured meats and hams have shown that the organism will survive the entire process, even when present at a low level of contamination. Thus, sanitation rather than an inhibitory curing process probably accounts for the low incidence of outbreaks from cured meats.

Staphylococci are the most common cause of food poisoning outbreaks from meats. A method recently developed by the Food and Drug Administration for the in vitro measurement of the staphylococcus enterotoxin is being used to study the effect of curing agents and temperature on toxin production.

In the contract project at Iowa State University on the relationship of fungi to the development of flavor, numerous fungal cultures have been isolated from hams exhibiting desirable flavor. Studies are now underway in which hams are being inoculated with these cultures to determine their effect on flavor under controlled conditions.

A P.L.-480 project has recently been negotiated with the Central Institute for Nutrition Research in Zeist, to investigate the effect of enterotoxins of microorganisms on protozoa. The aim of this research is to develop a sensitive assay system that will permit the rapid estimating of microbial enterotoxins in foods.

#### B. Product Stability Studies.

The isolation of free carbonyl compounds in the determination of flavor changes and deterioration in meat and meat products is important because of the major effect such compounds exert. In lipid oxidation, potential carbonyl compounds may be enormous compared with the free carbonyls, and the precursors and capacity for change complex and sensitive. Comparisons of quantitative methods for isolation of aldehydes showed wide differences in amounts separated from oxidized lard. The Girard T method, which was developed in this research, came closest to a quantitative isolation of free aldehydes. In this work a new, highly unsaturated class of aldehydes was isolated.

Studies have been made on relationships of specific aldehydes to rancid flavors. Judges were able to describe the presence of individual aldehydes of various classes in amounts ranging from 2 to 15 ppm. The contribution of an aldehyde to rancidity became greater with increasing chain length up to C<sub>10</sub>. The volatile aldehydes of a rancid lard contained individual aldehydes totaling only 1.1 ppm. This may indicate a powerful additive effect or the influence of other compounds. The characteristic aroma and flavor of lard



disappeared prior to the detection of off-flavors due to oxidation.

Preliminary experiments suggested that differences in stability of pork, beef, and lamb fat might not be due entirely to composition variation but to other factors such as glyceride structure. Back and leaf fat from lines of lard- and meat-type hogs have been processed for study of related variations in ketoglycerides, composition, and stability.

Under the grant at Florida State University, new methods have been developed for the determination of total meat pigments, percentage of metmyoglobin, and the enzymatic reduction of metmyoglobin. Enzymatic pathways in meat for the reduction of atmospheric oxygen and metmyoglobin are being studied.

In studies under the contract at the University of Missouri, on progressive changes in frozen and precooked frozen meats related to flavor stability, early results showed that heating beef caused increases in adenylic acid and decreases in inosinic acid. Nucleotides, other than adenylic acid, increased during storage at 0°F. Quick thawing of meats stored at 0°F. lowered the content of reducing sugars.

Under the P.L.-480 grant at the University of Helsinki, changes in lipid composition of dry sausages during ripening are under study. These are sausages produced by fermentation and, although similar to dry sausages, are produced in a shorter time. Early results show that oleic acid content decreases during the ripening period. This may be due to changes in glyceride structure or microbial effects. Current work is devoted to studying the lipolytic bacteria found in the sausages and to further investigations of the nature of the lipids present and the manner of their breakdown.

### C. Meat Composition and Quality Research.

Investigations of meat animal muscular structure were designed to ultimately lead to improved and enhanced tenderness and juiciness. Myosin and actomyosin, the principal proteins, were isolated, purified, and characterized; and facts pertinent to meat protein analyses were established. Related work, conducted by contract at Louisiana State University, dealt with the histochemistry and biochemistry of connective tissue. Tentative evidence for a relation of specific protein fractions to tenderness was established, and methods were developed for analyses of mucopolysaccharides and mast cell counts.

The research aimed at the improvement and enhancement of color in cured meats involved the isolation of pigments (myoglobins) from beef, lamb, and pork and investigation of their reactivity in meat-curing. Evidence obtained of the relative instability of pork nitrimetmyoglobin may lead to a better understanding of problems in pork-curing. The heat denaturation of the protein components of cured pork was investigated to develop a method for accurately determining the temperature to which meat products have been heat-processed; such a method would have special applications by the Meat Inspection and Animal Inspection and Quarantine Divisions, as well as by processors. Three proteins were isolated having heat denaturation rates that should be useful in

designing the accurate method needed.

Investigations of the manufacture of emulsion-based sausage, such as frankfurters and bologna, were initiated with special attention to accelerating the process through a better understanding of the effect of increasing temperatures above those conventionally used. Changes detected in proteins are being correlated with processing temperatures.

In a P.L.-480 project at the Low Temperature Research Station in England, Dr. S. M. Partridge has investigated the fundamental chemistry of some of the connective tissue proteins. Dr. Partridge isolated and described the structure of a chondroitin sulphate protein complex of bovine cartilage. This is an interesting compound which consists of a protein core surrounded by long, straight, polysaccharide chains. Another compound of connective tissue isolated and described was a sialomucoprotein. Dr. Partridge also studied the effect of proteolytic enzymes on these compounds and showed how they were hydrolyzed.

The natural reducing substances in pork tissue which may contribute to the color fixation reaction in meat-curing are being studied under a P.L.-480 grant by Dr. A. McM. Taylor and his associates at the British Food Manufacturers Research Association at Leatherhead, England. The work so far has been concerned with the isolation of pork muscle mitochondria and a study of the enzyme kinetics of the reactions between myoglobin and nitrite. Results indicate that normal cellular metabolic pathways are capable of reducing nitrite to provide nitric oxide under the anaerobic conditions obtaining during curing. The role of mitochondria, mitochondrial enzymes, and other cell constituents is being studied.

In a new P.L.-480 project at Chung Hsing University in Taiwan, Professor Chen You Kong is investigating the production of new types of meat products by a technique similar to drying. Fat is an excellent heat transfer medium for preparing meat products, and our war-time experience with meat dehydration leads us to feel that novel fried meat items may be developed through this research.

#### D. Meat Flavor and Smoke Composition Research.

The aroma of raw meat, while distinctive, is not particularly attractive for human consumption. After proper processing (cooking, grilling, broiling), a desirable odor develops. Precursors of the odor are present in the meat and, through a series of chemical reactions, give rise to the volatile components associated with the meat aroma. Since the aroma precursors are water-soluble, they are extracted from ground, raw meat. The proteins and large molecular weight material are removed by dialysis; the aroma is associated with the small molecules. The extract is separated by ion exchange fractionation on various resins. A fraction containing amino acids and hypoxanthine has been obtained which has a distinct meaty aroma. Sixteen amino acids, including the dipeptides anserine and carnosine, have been determined quantitatively in this fraction. The removal of hypoxanthine



results in alteration in the aroma. A number of tests, including the specific glucose oxidase assay for glucose, and gas-liquid chromatography of the tri-methyl silyl derivatives, indicate the absence of sugars in the fraction with a meaty aroma. While studies are underway to further confirm this finding, it is of interest because it has been assumed that meat flavor and aroma are due to a "browning reaction" involving amino acids and sugars.

More than 200 compounds are known to be formed by the thermal degradation of wood as used in the preservation of meat by smoking. Identification of the desirable components in good smoke will lead to improved techniques for their selective production. A laboratory scale smoke generator, heated by electricity, has been constructed in such a way that the rate of heating and the air flow can be controlled, temperatures recorded at various points in the system, and samples of smoke obtained in appropriate traps. Analysis of the trapped smoke solution by the use of the gas-liquid chromatograph and infrared spectroscopy has led to the identification of a number of phenols and neutral compounds. While all the components have not yet been identified, the appearance of components as separated by gas-liquid chromatography permits tentative comparisons among smoke preparations. Commercial liquid smoke preparations are being introduced and are being well accepted by the industry. The solutions vary considerably in odor and taste, and gas-liquid chromatography examination shows marked differences in composition between the natural and artificial, or imitation, smoke solutions. Recent literature reports have indicated the possibility of the presence of carcinogens in smoke and smoke solutions, and work is being planned to investigate the significance of these compounds.

Research under the contract at Michigan State University has already developed a smoked ham base which is now being incorporated into commercially feasible products. Other products are in the process of development.

A P.L.-480 grant by Dr. J. Tilgner at the Technical University at Gdansk, Poland, is devoted to studying the antioxidants of smoke. To date, smoke fractions have been isolated, and their antioxidant properties are under study.

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ANIMAL FATS AND OILS - INDUSTRIAL UTILIZATION  
Eastern Utilization Research and Development Division, ARS

Problem. The 4-1/2 billion-pound-per-year output of inedible fats is one of the major products of the livestock industry. It also is one of major concern, because while production of animal fats has more than doubled in the last 15 years, its principal outlet (in soap) has declined sharply, and is still declining.

The best answer to the question of what to do with huge amounts of fats is to find new uses through utilization research. Already utilization research has played a leading role in finding new uses for over 1 billion pounds of animal fats, and thus helped retain markets for fats. Use of fat in animal feed which was developed through research, has now become the number one domestic use of inedible fats. There is need, however, for new uses not merely to retain or defend markets, but to expand them, and to upgrade the value of animal fats. The organic chemical industry presents a good opportunity for expanded markets, producing as it does a multitude of products--polymers, plasticizers, insecticides, herbicides, lubricants, paper chemicals--totaling 10 billion pounds. Animal fats possess "built-in" properties which make them potentially useful as raw materials to the chemical industry, but research must be done to realize this potential.

An increase of 1 cent per pound in the value of inedible animal fats would provide an additional revenue of \$40 million of the livestock industry. This additional revenue will help the industry and growers in the same way as revenue from other animal products and by-products.

The attainment of an increase in the monetary returns from livestock requires both applied and basic research. Applied research is the forerunner of commercial practice and is an indispensable element in successful development. But applied research is based on the foundation of fundamental knowledge that is acquired through basic research, and represents the exploitation of this fundamental knowledge. The supply of fundamental facts about animal fats; composition, methods of separation of constituents, preparation of chemical derivatives of constituents and determination of their physical and chemical properties; must be maintained and expanded if applied research is to be most effective and fruitful. The need for basic research has been pointed out by the Commission on Increased Industrial Use of Agricultural Products, the National Agricultural Research Advisory Committee and by other responsible groups.

USDA AND COOPERATIVE PROGRAM

The Department has a broad program of basic and applied research at Wyndmoor, Pennsylvania, and at additional locations where contract and grant research is being carried out involving chemistry and physics, aimed at developing new and improved products from fats for use in industry. Total professional man-years (p.m.-y.) are 53.8. Of this, 11.9 p.m.-y. are devoted to studies on



chemical composition and the physical and chemical properties of animal fats at Wyndmoor. Studies involve fatty acid composition of animal fats using the latest advances in chromatography and other techniques; intra- and inter-molecular structure of pure compounds and derivatives and factors that influence development of off-flavors in fatty foods. Research is underway at Villanova University, Villanova, Pa. with two contracts on special inter-relationships within triglyceride molecules and on x-ray investigation of triglycerides and involves 0.7 p.m.-y. each. A research grant involving 1.0 p.m.-y. at Storrs, Conn. provides for the synthesis of pure glycerides. Additional research sponsored by the Department under P.L. 480 grants is now in progress at the University of Madrid, Spain, on the preparation of cocoa butter substitutes from animal fats, and at the Technical University, Gdansk, Poland, on a study of the kinetics and thermodynamics of fat autoxidation.

Research on improved polymers, plastics, resins and lubricants involving 16.3 p.m.-y. at Wyndmoor is conducted on the synthesis of organic compounds and the preparation of new products for use as plastics, plasticizers and lubricants. A research contract with the University of Arizona at Tucson involving 0.2 p.m.-y. deals with use of products derived from animal fats to synthesize plastics and other polymeric materials. In the current program an aspect of animal fat research is a contract with U. S. Industrial Chemical Company, New York on "Ethylene copolymerization with unsaturated fatty acids and gum naval stores." EU shares the effort to the extent of 0.4 p.m.-y. in cooperation with SU. Additional research underway at the University of Aix-Marseille at Marseille, France with P.L. 480 funds concerns preparation of hydroxylated derivatives of animal fats for use in industrial products such as plastics and lubricants.

At Wyndmoor 9.4 p.m.-y. are being devoted to research on development of improved synthetic detergents based on animal fats, which includes preparation, testing of detergent power, and measurement of biodegradability of  $\alpha$ -sulfo fatty acids and their esters, tallow alcohol sulfates and other fat derived materials. A research contract with Lehigh University, Bethlehem, Pa. involving 0.5 p.m.-y. deals with interfacial absorption characteristics of fatty acids. The high pressure hydrolysis of animal fats to alcohols without simultaneous chain saturation will be investigated under a research contract with Swift and Company, Chicago, Ill. Additional research sponsored by the Department under a P.L. 480 grant is now in progress at the University of Bombay, Bombay, India on the preparation and properties of long chain sulfated monoglycerides.

Exploratory investigations of new chemical derivatives of animal fats for use as chemical intermediates for industry involves 10 p.m.-y. at Wyndmoor. A research contract on the chemical and physical characteristics of organic peroxides involving 0.7 p.m.-y. is in progress at the University of Pittsburgh, Pittsburgh, Pa. A research grant with the Hormel Institute of the University of Minnesota at Austin, Minnesota involving 0.6 p.m.-y. provides for the investigation of the ozonization of animal fats.

## PROGRAM OF STATE EXPERIMENT STATIONS

A limited program of work directed to the utilization of fats and oils is in progress at the State Stations. More complete knowledge of the molecular structure and composition of the component glycerides is being developed and related to flavor stability. Chemical transformations are under investigation to minimize polymerization and conjugation during isomerization processes leading to industrially modified fats and oils. Studies continue on the nutritional quality of fats to assess the vitamin E content of diets low in cholesterol and poly-unsaturates. One research group is exploring the role of lipids in maintaining the beat of heart cells in vitro. In another study the metabolism of trans fatty acids, which occur in most edible hydrogenated fats, is being evaluated. Still another study deals with the chemical stability of frying fats, including lard, vegetable shortenings, and mixture of animal fats with hydrogenated vegetable oils to withstand repeated heating, re-use, and storage.

The role of antioxidants and pro-oxidants is being investigated. Other research is directed toward identification of the components responsible for the flavors of auto-oxidized fats and the factors affecting their production. A precise quantitative method of analysis involving gas chromatography has been developed for C<sub>6</sub> and C<sub>22</sub> fatty acids. Evidence for auto-oxidized flavor components of fats points to such aldehydes and ketones as cis, cis-3, 6-nonadienal and vinyl ethyl ketone. Synthesis of glycerides with controlled degrees of unsaturation have been worked out for testing changes in blandness.

Basic studies on minor constituents of animal and vegetable fats such as sulfo-lipids, phospho-lipids, and steroids are underway. Applied work to convert low grade fatty materials into industrially valuable products has led to a potentially commercial method for producing the more stable elaidic acid from oleic acid. Related studies on the trans-isomerization of ethyl linoleate are being pursued.

A total of 2.5 professional man-years is devoted to research on industrial utilization of animal fats and oils at the State experiment stations.

## PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition, Physical Properties and Autoxidation.

1. Purification of lipids and determination of their structure. Through the use of polarized infrared spectroscopy, additional information was obtained on the structure of thin crystalline films of methyl esters of fatty acids and alkyl sulfonic acids; the data permit assignment of absorption bands in these compounds. Nuclear magnetic resonance spectroscopy was applied successfully to investigate the intramolecular hydrogen bonding of a model compound in polar solvents. Dielectric measurements were used to determine some thermodynamic properties of esters of fatty acids. X-ray diffraction methods have been applied to the determination of free urea in urea adducts of ethyl stearate.



The specificity of pancreatic lipase for the hydrolysis of the 1,3 ester groups was previously shown to be absolute and the technique to be useable as a criterion of purity for di- and triglycerides. A semi-micro hydrolysis technique has been developed in which the hydrolysis products are isolated by thin-layer chromatography and the fatty acid mixtures analyzed by GLC. By means of the semi-micro lipase hydrolysis technique, the glyceride distribution of depot fats from a series of animals was determined and a comparison was made of the glyceride distribution of normal lard and a randomly inter-esterified lard. Whereas lard has typically over 80% of palmitic acid in the 2 position, the randomly interesterified lard showed large decreases in the content of 2-palmitoyl triglycerides and large increases in the amount of symmetrical and unsymmetrical diunsaturated glycerides.

Under a P.L. 480 grant at the "Juan de la Cierva" Foundation for Applied Research, Madrid, Spain, some progress has been reported in the development of suitable methodology in glyceride analysis for use in research on preparation from animal fats of substitutes for cocoa butter.

An unsymmetrical di-acid triglyceride has been successfully synthesized and determinations by x-ray intensity data and by unit cell dimensions have begun. A computer program has been prepared to analyze the x-ray data. Data of this kind will permit the exact determination of the structure of a single crystal.

Computers have been employed to calculate the effect of small changes in bond angles on intramolecular hydrogen bonding. These computations have demonstrated that the geometry of a large number of isomers of a molecule can be determined provided a high speed computer is available. A computer program has been developed for application to the calculation of spatial inter-relations of triglyceride molecules.

2. Autoxidation. The autoxidation of methyl linoleate in emulsion has been studied in the presence of both cupric ion and its chelates with arginine and histidine. The results indicate that careful control of pH in an aqueous-fat system may be used to inhibit or accelerate the autoxidation of the fat. In the system so far investigated the pH range of 6-7 appears to be optimum for suppressing fat autoxidation. In the presence of a model system simulating oxidase activity the rate of oxygen uptake at pH 5.7 of a 0.1 M methyl linoleate emulsion was increased approximately 25 times. The model system contained ascorbic acid, ethylenediaminetetraacetic acid and ferric ions. As the ascorbic acid was destroyed the rate of oxygen uptake decreased from 25 to 4 times that of the control, indicating that ascorbic acid, often used as an anti-oxidant, may function as a pro-oxidant.

## B. Improved Polymers, Plastics, Resins and Lubricants.

1. Polymers, plastics and resins. Internal plasticization was evaluated on copolymers of vinyl esters of cyclic and polychloro fatty acids with vinyl chloride. The lowest brittle point and most effective plasticizing was obtained with vinyl dichlorobehenate as the comonomer, followed in

effectiveness by vinyl stearate and vinyl dichlorostearate. Confirmation of internal plasticization of vinyl chloride by these vinyl comonomers enhances their prospects for commercial use.

Rigid urethane foams were readily prepared from polyols made by the reaction of 9,10-dihydroxystearic acid, either the erythro- or threo-isomer, with propylene oxide. Whereas all of the ethylene oxide adducts of erythro-dihydroxystearic acid, as well as the di- and octa-oxyethylates of the threo-isomer, were inconveniently solid or semi-solid at room temperature, the propylene oxide adducts (1, 2, 4, 6 and 8 oxypropylene units) to either erythro-dihydroxystearic acid or the threo-isomer are liquids throughout both series. This feature, and the slower creaming times of propylene oxide adducts in reaction with isocyanate than ethylene oxide adducts, provides 9,10-dihydroxystearic acid derivatives which are convenient for use in 2-stage foam preparation and enhances the possibility of their commercial utilization. Measurement of the physical properties of the two rigid urethane series embodying oxypropylated derivatives of 9,10-dihydroxystearic acid has been completed except for the determination of compressive strength. The foams were found to contain over 80% closed cells, with densities ranging between 1.6 and 1.9 lbs./ft.<sup>3</sup>.

Periodate cleavage of monoepoxidized methyl linoleate by two different versions of the periodate method indicate that there is no significant selectivity in the epoxidation reaction. The 9,10- and the 12,13-double bonds of methyl linoleate are epoxidized with equal ease, giving a product containing substantially equal amounts of the unsaturated epoxy derivatives, methyl coronate and methyl vernolate. Demonstration for the first time of methyl coronate as a synthetic product was made possible by the new variations of the periodate cleavage reaction.

In P.L. 480 supported research at Universite d'Aix-Marseille, Marseille, France, conversion of brominated fatty compounds to allylically hydroxylated derivatives was completed, the results were reported in two publications, and research was initiated on: allylic hydroxylation of unsaturated fatty acids with selenium dioxide; oxidation of allylically hydroxylated oleic acid to  $\alpha,\beta$ -unsaturated ketones; and oxo addition of carbon monoxide and hydrogen to double bonds. For GLC identification of expected products, model branched long-chain derivatives were prepared from carboxystearic acid made by the ERRL method.

Evaluation was completed of the copolymers of N-n-butyl, octyl and octadecyl acrylamide with acrylonitrile with respect to torsional stiffness, temperatures and tensile properties. Tensile tests, which measure the effect of large chain deformations by relating stress to strain up to the break point, showed failure at low strain, characteristic of brittle materials. This affect was most pronounced with the octadecyl acrylamide. At temperatures greater than 100°C. where presumably the crystalline domains had melted, polymers were tough, resilient and flexible, the improvement in properties increasing with increase in amide chain length. Data obtained from the



evaluation of these copolymers indicate that long chain unsaturated acrylamides, such as oleyl acrylamide, or long chain tertiary amides such as N-N-di-N-octadecyl acrylamide, will have a higher internal plasticizing efficiency and impart useful properties to acrylonitrile copolymers.

2. Lubricants. The dialkylphosphono adducts of unsaturated fatty acid esters or triglycerides from animal fat sources have been prepared and evaluated for use as lubricants or lubricant additives. From an economical viewpoint the naturally occurring triglycerides are the most attractive olefinic starting materials. However, when the phosphorus-containing triglycerides are prepared by the free radical addition of dialkylphosphonate to their olefinic centers by techniques entirely satisfactory for addition to monounsaturated esters, the time necessary for a complete reaction was increased by a factor of 5 to 6, to overcome this disadvantage a study was made of the time-temperature relationships for the reaction. It was found that an increase in the reaction temperature from 105°C. to 125°C. reduced the time for complete reaction 10-fold, or from 30-40 hours to 3-4 hours. When compounds prepared in this improved fashion were submitted to lubricant evaluation by anti-wear and extreme-pressure tests, they showed no deterioration in physical properties.

The preparation and lubricant evaluation of the products of the addition of dialkylphosphonates to unsaturated fatty esters and triglycerides was extended to the diesters of ethylene glycol. The products obtained are low melting solids with lubricant properties comparable to those found for the derivatives of triglycerides and monoesters.

Lubrication tests indicate that phosphorus-containing compounds derived from fats and oils resist wear, extreme pressure, and oxidation when used as base oils or as additives to both petroleum lubricants and synthetic diester lubricants.

### C. Improved Synthetic Detergents.

1. Soap-detergent combinations from inedible animal fats. The esters of  $\alpha$ -sulfo fatty acids have markedly different properties; the pelargonates are primarily excellent wetting agents, the myristates, palmitates and stearates are lime soap dispersing agents; and these differences can result in specialized uses for individual esters. Eleven of the esters were prepared in a high state of purity and delivered to a contractor for measurement of physical-chemical characteristics. Preliminary information of the effect of pH and added electrolyte on surface tension values, and preliminary heat of wetting data have been obtained. The relation of interfacial absorption characteristics with structure of a compound and its performance as a detergent or wetting agent may be a guide to the further development of surface active agents and detergents from animal fats.

A series of monovalent and divalent metal salts of the alkyl esters of  $\alpha$ -sulfo fatty acids were prepared for a study of structure-property relations.

Although all of the monovalent salts are sufficiently soluble in water, the potassium salts are the least soluble. Calcium and magnesium salts from gelatinous aqueous dispersions at higher concentrations, but they are readily soluble in organic solvents. Of 14 sodium salts evaluated for detergency, foaming, wetting, or lime soap dispersing properties, sodium methyl  $\alpha$ -sulfopalmitate gave the best all around performance. All the esters are biodegradable.

Other compounds prepared are the ether alcohol sulfates and sodium alkene-sulfonates. The latter were prepared by sulfonation of tallow-derived  $\alpha$ -olefins. Sodium alkenesulfonates resemble sodium oleyl sulfate; they are readily soluble in water and have good detergent and surface active properties.

Ether alcohol sulfates of the formula  $\text{ROCH}_2\text{CH}(\text{OSO}_3\text{Na})\text{CH}_3$  were prepared by the oxypropylation and sulfation of saturated long carbon chain primary alcohols. The ether alcohol sulfates are excellent detergents and lime soap dispersing agents, being more readily soluble and more generally useful than saturated tallow alcohol sulfates. Both the ether alcohol sulfates and the sodium alkenesulfonates are easily biodegradable.

2. Biodegradability. The ease of biodegradation of tallow alcohol sulfates and esters of  $\alpha$ -sulfo fatty acids were compared with linear (LAS) and branched-chain (ABS) alkylbenzenesulfonates under aerobic conditions representing the operation of an activated sludge sewage disposal system and under conditions representing anaerobic digestion in municipal sewage systems, septic tanks and cesspools. Tallow alcohol sulfates in particular, and certain esters of the  $\alpha$ -sulfo fatty acids, are more readily tolerated than either linear or branched chain alkyl benzene sulfonates under both aerobic and anaerobic conditions of biodegradation.

A new method of evaluation of biodegradability based on chemical oxidation demand (COD) is under investigation. Whereas the currently used methylene blue test indicates degradation of the detergent to the point of loss of surface active properties, the COD measurement indicates extent of metabolism by microorganisms. From preliminary tests it appears that surface active properties are lost long before COD values reach a minimum.

#### D. New Chemical Derivatives.

1. Reactions and Reaction Mechanisms. Direct free radical addition of addends to unsaturated acids may lead to a variety of versatile and useful chemical derivatives. Investigations of the thermal free radical addition of acetic acid, acetic anhydride and ethyl cyanoacetate to methyl undecylenate and methyl oleate has been completed. A similar study on aromatic aldehydes has been initiated, but preliminary results indicate this reaction to be a complex one.

Improvements in the synthesis of diisopropenyl esters of dicarboxylic acids has improved the yield to about 60%, double that obtained initially.



Deuterium-labeling studies are being employed to investigate the mechanism by which gamma-stearolactone is formed in perchloric acid isomerization of oleic acid. The products are separated by column chromatography and deuterium analyses carried out on the individual products. Mass spectrometry is being used to determine the position of deuterium in the products. Results obtained support the hypothesis that the formation of gamma-stearolactone is mainly due to a series of intermolecular reactions of carboxyl with olefin to form ester (a dimer), a reaction which is reversible and results in double bond migration along the hydrocarbon chain. Ultimately the double bond reaches the vicinity of the carboxyl group on the same molecule, whereupon an intermolecular esterification results in the lactone formation.

The isomerization of oleic acid to gamma-stearolactone with sulfuric acid, the classical reagent, was reviewed thoroughly and systematically. None of the reactions produced gamma-stearolactone in yields comparable to those obtained from the perchloric acid isomerizations.

A program on the use of gamma-stearolactone as a chemical intermediate has been initiated. Alkali-fusion of gamma-stearolactone gave good yields of gamma-ketostearic acid.

The thiocyanation of methyl oleate was restudied with cis and trans-9-octadecene as model compounds for clarification of the mechanism. Nine distinct products of the reaction were obtained by column chromatography and counter-current distribution.

2. Properties and structural characteristics of organic peroxides. Organic peroxides derived from animal fats are easily and inexpensively prepared, and they are labile compounds which undergo a wide variety of organic reactions. Basic information on the structure of these peroxide derivatives and thermodynamic data will aid in the development of reactions for converting peroxides of fats and their derivatives to commercially useful chemicals.

Results of the structure refinement of dibenzoyl peroxide obtained so far reveal an unusually interesting thermal behavior of the molecules in the solid state. The space group of the crystals is also compatible with a single enantiomorph suggesting that one single crystal will contain molecules of the same sense. The study of optical rotatory properties of benzoyl peroxide is contingent upon success in growing appropriate single crystals. This work is being conducted at the University of Pittsburgh under a research contract.

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HIDES, SKINS AND LEATHER - PROCESSING AND PRODUCTS  
 Eastern Utilization Research and Development Division, ARS

Problem. To maintain the utilization of animal hides and skins at a profitable level there is need to find new products and processes to provide outlets for about 11 million cattlehides that are now available in excess of domestic needs. The foreign markets that currently absorb these surplus hides are also threatened by the increased hide production and decreased per capita use of leather (the principal outlet for hides) that have dislocated U.S. markets and caused prices to drop so precipitously in the last 10 years. To meet this problem there is need for upgrading the quality of raw hides and skins, for reducing the costs of producing leather, and for developing new and non-conventional products from collagen. To achieve these objectives research is needed to develop improved curing processes and agents, more effective control measures for (ante mortem) defects such as grubs, brands and parasite damage, and improved methods of take-off. Fundamental research is needed on the composition of hides to provide basic information on the chemical, physical and physical-chemical properties and reactions of collagen and other hide components for use in studies on chemical modification and on the development of new and improved products and processes. Development of new, more rapid and economic processes for curing, handling, unhairing and tanning hides is needed to reduce the cost of producing leather. There is also need for research on the chemical modification of hide proteins to develop leather products with such improved "built-in" properties as increased resistance to wear, scuffing and deterioration from perspiration, enhanced washability, dry-cleanability and improved dyeability. There is also need for research on the physical and chemical properties of collagen to obtain information for use in dispersing and regenerating the fibrous structure without degrading its unique properties for developing nonconventional products that will provide new outlets and markets for hide proteins, with special reference to the field of edible products.

USDA AND COOPERATIVE PROGRAM

The Department is conducting a broad program of basic and applied research on hides, skins and leather at Wyndmoor, Pennsylvania, and at additional locations where contract and grant research is being carried out; this involves physicists, chemists, biochemists, microbiologists and leather technologists.

The Federal scientific effort devoted to the over-all program totals 29.8 professional man-years, as follows:

(a) Chemical and physical properties and structure of hides and collagen involve 8.6 p.m.-y. at Wyndmoor. These investigations encompass exploratory research on cattlehide components and basic research on the chemistry of collagen. One line of investigations is concerned with the isolation and identification of cattlehide components, their chemical and physical properties, and their organization within the hide structure as related to leather properties. The second part of the program deals with the forces that control

the stability and reactivity of collagen and the factors responsible for the unique physical properties of leather. Of special interest are the physical-chemical properties of collagen, its soluble components and its reaction products with modifying chemical agents. The University of Cincinnati, Cincinnati, Ohio, is conducting contract research on the noncollagenous proteins of cattlehides involving 0.4 p.m.-y.

Under a grant with Northwestern University, Evanston, Illinois, 0.9 p.m.-y. is being spent to study the physical properties of collagens.

In addition, research sponsored by the Department under the P.L.-480 program is in progress at: (1) University of Turku, Finland, on the fractionation of gelatin and soluble collagen; and (2) Central Leather Research Institute, Madras, India, on the reaction of polyphenolic tanning compounds with hide proteins (collagen); on the hydrothermal shrinkage of collagen and leather; and on the comfort properties of shoe leathers.

(b) Chemical modification of hides involves 9.0 p.m.-y. at Wyndmoor. This program is concerned with research on the reactivity of hide proteins with various organic and inorganic chemicals in the development of new products intended for specific uses.

In addition research sponsored by the Department under the P.L.-480 program is in progress at the British Leather Manufacturers Association, Surrey, Great Britain, for the study of chemically reactive compounds to improve leather stability.

(c) New and improved processing involves 8.6 p.m.-y. at Wyndmoor. Research is aimed at developing better methods for unhairing animal hides and skin, for imparting deterioration resistance to leathers, and for processing hides into leathers possessing special properties. The University of Cincinnati, Cincinnati, Ohio, is conducting research on the abnormalities of leather characterized by a depleted mushy texture involving 1.4 p.m.-y., and the Midwest Research Institute has initiated studies on the dispersion of collagen for the development of new products involving 0.7 p.m.-y.

Additional research under P.L.-480 grants is in progress at: (1) Central Leather Research Institute, Madras, India, on the interrelation of hide quality with the rate of tanning and efficiency of tanning and on the rapid tannage of sole leather; and (2) Leather Research Institute, T.N.O., Waalwijk, Holland, on kinetics of chrome tanning.

(d) Utilization of animal protein residues involves research in cooperation with the National Renderers Association, who support one Senior Fellow and one Junior Fellow at Wyndmoor, Pa., to conduct basic studies on the evaluation of meat and bone meal fractions of residues from fat rendering operations. The Department of Agriculture participates in this program to the extent of supplying supervisory and research leadership amounting to 0.2 p.m.-y.

## PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

State stations reported no research in this area.

## PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical and Physical Properties and Structure of Hides and Collagen.

1. Chemical and physical properties. Observations have been made on the thermal behavior of collagen and leather through the use of differential thermal analyses. Information obtained not only provides a better understanding of the basic energy changes involved when hide materials undergo a change but are of immediate importance to applied problems involving the manufacturing (processing) of leather.

The mechanical properties of leathers have been investigated by means of a nondestructive dynamic test procedure. Comparative measurements of physical characteristics were made over the entire area of intact sides of leather which revealed a pattern of the variability of the properties within a side.

Continued progress has been made under a P.L.-480 grant at the University of Turku on the specific properties of collagen and gelatin that are applicable to the development of expanded uses of animal hides and skins. Starch gel electrophoresis has proved to be a powerful tool in the separation of most of the components from denatured collagen. Application of linear temperature gradient in the gel sheets has demonstrated how denatured collagen subunits change to the collagen-fold in this order with decreasing temperature:  $\beta_{11}$ ,  $\beta_{12}$ ,  $\alpha_1$ ,  $\alpha_2$ . Studies have revealed that when collagen is hydrolyzed with pepsin, that two distinct units are split from the bulk of the collagen. Their source needs to be determined. The P.L.-480 grantee at the Central Leather Research Institute, Madras, India, has reported further progress on the mode of reaction of polyphenolic compounds with collagen. Purified tannin derived from mangrove penetrates the hide faster and gives higher hydrothermal stability than crude extracts. This has prompted the fractionation and evaluation of other tannins to establish the universality of this property.

Under a P.L.-480 project the Central Leather Research Institute, Madras, India, is studying the effect of tannery processes, such as liming, bating, pickling, tanning, fat-liquoring, etc. on the shrinkage temperature ( $T_s$ ) and the accompanying dimensional changes. Specimens from each stage showed appreciable loss in area when the shrinkage temperature was exceeded. The change was least for limed stock; all of the others showed about a 10% loss, while pickled stock showed the most pronounced change of 30 to 40%. This Institute has recently initiated work under another grant on the factors affecting the comfort of leather apparel and footwear.

2. Microscopy. Progress has been made in the microscopy of the structural features of hides and skins and has yielded significant information on the disorganizations in the architecture associated with such defects as



veininess and mushy texture. Microscopy with polarized light and with phase systems offers a new dimension of observation and has revealed details of structure not seen under ordinary procedures. Use of ultrathin wedges has shown that structural features can be observed both under visual and electron techniques. This overlap makes possible a continuous range of magnification from a few powers up to 100,000 times in controlled steps so that identity of features is not lost. With these improved techniques for determining hide architecture more progress toward identifying the chemical and physical causes of hide defects can be made and the economic losses caused by them eliminated.

3. Collagen dispersion. Viscosity measurements on preparations of calfskin collagen solubilized in inorganic buffers has demonstrated a "non-Newtonian" behavior. Correction for this effect may more than double the presently accepted value for the intrinsic viscosity of collagen. The fundamental information resulting from this study should enhance the understanding of the physical chemistry of solubilized collagen and presents a new point of view regarding the viscometry of systems composed of large macromolecules. Since the continued profitable utilization of animal hides will depend to some extent upon the development of new products from collagen, information on its behavior toward dispersing agents and to changes in influence of ions will aid in the development of a process for the dispersion and reconstitution of collagen and may lead to new items containing its characteristic properties.

Behavior of collagen and gelatin in nonaqueous systems are under investigations at the Northwestern University School of Medicine. Physical measurements on the interactions of various nonaqueous solvent pairs indicate the presence of strong hydrogen bonding. These solvent pairs are being utilized to study the behavior of synthetic polypeptides of known composition and subsequently will be used for solubilizing collagen.

4. Effect of hide components on properties. A study of the distribution of the lipid classes in each of five stratigraphic layers for eleven areas whose combined area totaled over two-thirds of the area of a steer hide has shown that the variability of the lipid content of a hide is more complex than expected. Each of the five lipid classes has its own pattern of variability both areawise and through the thickness of the hide. This is one indication of the extreme complexity of the raw material used by tanners.

In contract work at the University of Cincinnati it has been shown that the noncollagenous proteins are extracted to varying degrees from hides by brines of different concentrations. This may explain the many conflicting reports in the trade about the leathermaking qualities of commercial brine-cured hides. A 1% brine appears to be the most efficacious extractant from the standpoint of ease of use and quantity of proteins extracted. Hides extracted with different brines are being tanned into shoe upper leather and the data obtained on the changes produced by differences in brining operations will be useful in developing recommendations for improving the quality of brined hides.

## B. Chemical Modification.

1. Improved water repellency for leather. Previous reports have outlined progress in this area. A large-scale test has now been made with 12 commercial sides. It has been found that retannage of chrome leather with glutaraldehyde and lubrication with an alkenyl succinic acid improves the efficiency of subsequent water repellent treatments with silicone, Scotchgard, or Quilon. The most important feature of this development is the novel process for applying the alkenyl succinic acid to leather from an emulsion. These studies have demonstrated that smaller amounts of water repellent can be used and still retain effective water repellency.

2. Mannich reaction. The amount and kind of reactive groups present in hide protein limit the scope of its chemical reactivity. However, the Mannich reaction, which involves interaction between amine, formaldehyde, and a compound with an active hydrogen, can broaden this scope of reactivity. Hide protein (source of amino groups) was treated with formaldehyde and a wide variety of compounds with active hydrogen. The most promising of the latter was malonic acid. Studies of model system revealed that treatment of glycine,  $\beta$ -alanine, and  $\alpha$ -aminobutyric acid with formaldehyde and malonic acid gave complex unresolved mixtures. However  $\delta$ -aminovaleric and  $\epsilon$ -aminocaproic acid both gave insoluble and apparently pure compounds whose structures (through infrared and NMR spectra) are indicated to be  $(\text{HO}_2\text{C})_2\text{C}(\overline{\text{CH}}_2\text{NH}(\text{CH}_2)_n\text{CO}_2\text{H})/2$ , where  $n = 4$  and  $5$ , respectively.

3. Aldehyde leathers. A spectrophotometric method for determination of glutaraldehyde in solution, based on the 2,4-dinitrophenylhydrazone derivative, was developed. This procedure is rapid and specific for carbonyl groups and permits analysis for glutaraldehyde in the presence of many substances used in tanning of leather.

A method for the direct determination of bound glutaraldehyde in leather has been evolved. This is based on the discovery that hydrolysis of glutaraldehyde-tanned hide protein produces a substance absorbing at  $265 \text{ m}\mu$  in the ultraviolet region. From a study of glutaraldehyde balance it was shown that absorbance at  $265 \text{ m}\mu$  correlated with uptake of glutaraldehyde.

Early investigations demonstrated glutaraldehyde-tanned leather to have superior resistance to deterioration by perspiration and hot, soapy water. Washability, a property not enjoyed by leather, would enhance the utilization of hides and skins. A study on glutaraldehyde garment leathers showed the improved stability of this tannage to washing and offers considerable promise in stabilizing chrome leather. However other problems, such as wash-fast dyes, oils, and finishes, are confronted and need investigation. Research has begun on evaluating reactive dyes, such as the Procion dyes, in this application.

Studies have demonstrated the tanning ability of Dextraset UN under mildly alkaline conditions. This material, a cyclic derivative based on urea, formaldehyde, and methanol, produces a white leather. Further work is



necessary to define the optimum reaction conditions and to evaluate fully the properties of the leather. Other methylol derivatives have also been studied including those of imidazolidone, ethyl carbamate, urea, ethylene urea, and hydroxyethyl carbamate. In general the treatments with these methylol derivatives were similar to those with formaldehyde but with lower reactivity and shrinkage temperature.

The stability of hide protein can be increased by introduction of cross links through modification with polyfunctional reagents. Research under a P.L.-480 grant with the British Leather Manufacturers Research Association has been initiated to study mechanisms by which leather can be stabilized against deterioration from sweat and other chemicals. Exploratory studies with glutaraldehyde, formaldehyde, glyoxal, cyanuric chloride, acid chlorides, and a vinyl derivative indicates that glutaraldehyde is the most active cross-linking agent. Glutaraldehyde labeled with isotopic carbon was synthesized and used in studies on the mechanism of its reaction.

### C. New and Improved Processes.

1. Enzymic unhairing of hides and skins. It has not been possible to produce commercially satisfactory shoe upper leather from enzyme unhaired hides in the complete absence of the conventional alkaline swelling of the collagen. Lime has been used after enzyme unhairing to produce reasonably good leather in several instances, although the results have not been wholly consistent on all lines of leather in different tanneries. While the use of lime increased the sewage disposal problem it has been possible to eliminate the most objectionable constituent of an unhairing liquor, namely, the inorganic sulfide. Commercially acceptable sole and handbag leathers have been produced from enzyme-unhaired hides without the use of any lime. An industrial firm is now conducting applied research in this area as a result of our contributions. The project will be terminated this year.

2. New tanning processes. The use of glutaraldehyde is expanding rapidly to improve the perspiration resistance and alkali stability of leather. Special applications such as the tanning of woolskin hospital bed pads, cattle belly work glove leather and deerskin garment leather, the latter in cooperation with RAD, are under investigations. Tanners are now so well aware of the practical advantages of the glutaraldehyde tannage that they are finding applications and are even adding lines of leather new to them.

3. Abnormalities of leather characterized by a depleted, mushy texture. Continuing progress under contract with the Tanners' Council Laboratory of the University of Cincinnati has established that a hide defect known in the trade as "pulpy butts" is largely, although not exclusively, confined to heavy, plump Hereford hides. Spready Hereford and plump Angus hides are almost free of this defect. Histological sections revealed that there is a much more vertical "angle of weave" in the fiber structure of the weak areas. Analyses of strength data indicate that the stitch tear test may be as reliable as the tensile test in predicting incidence of defective leather. Also stitch tear could be performed with greater facility in a slaughterhouse.



4. A study of the kinetics of chrome tanning. This research is being carried out under a P.L.-480 grant at the Lederinstituut T.N.O., Waalwijk, Holland. Initial work on pure basic chromium sulfate solution shows that the amount of chromium retained by the hide increases continuously, but the effect of time decreases with decreasing chromium concentration. Basicity of the liquor is important only during the early stage of tanning. The pyridine method for determining ionic sulfate is unreliable for different basicities and time of ageing of the tanned leather. Acetone dehydration of the tanned hide powder arrested the changes and will be used at different time intervals to determine the different states of the chromium sulfate.

5. Relationship of hide quality to tanning rate. The Indian grantee of this P.L.-480 project has shown some interesting differences between hides taken from fallen animals and those that were slaughtered. The fallen hides gave a positive test for delayed cure by the gelatin film procedure. The fallen hides showed excessive veininess, blood occlusion and grain damage. No differences could be detected internally by microscopy. The chrome tanning rate was not greatly affected by the hide quality. However, finished leather from the fallen hides was downgraded.

6. Investigations on "red heat." The Stazione Sperimentale per l'Industria delle Pelli e delle Materie Concianti of Naples, Italy, has concluded studies under a P.L.-480 grant in the causes and prevention of "red heat" damage that occurs on salted American packer hides received abroad. Pilot scale tests by Armour and Company revealed that the four germicides evaluated did not completely prevent the development of red heat when added to the curing salt. Subsequent studies by the grantee revealed that the loss of germicidal potency was caused by the U.S. commercial practice of employing a mixture of used and new salt for curing. Laboratory tests proved that the excessive inoculum carried by the used salt exceeded the germicidal power of the chemicals employed.

#### D. Utilization of Animal Protein Residues.

Investigations supported by the National Renderers Association are concerned with evaluating the quality of meat and bone meals from fat rendering residues by characterization of the proteins present. Amino acid analysis of the numerous fractions prepared from representative meals has shown that: (1) all the globular protein fractions are similar; and (2) the gelatin or nondialyzable fraction from autoclaving contains many amino acids not derived from collagen. The presence of amino acids not originating from collagen or gelatin indicates that autoclaving to remove the gelatin also removes other protein fragments and cannot serve as a procedure for enriching the meals. Early in the year the Fellows resigned, and the National Renderers Association have asked that new personnel be recruited for continuation of the program.

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### III. MARKETING AND ECONOMIC RESEARCH

#### LIVESTOCK AND MEAT - MARKET QUALITY Market Quality Research Division, ARS

Problem. To insure that grades are a true measure of palatability, a better understanding of the relationship between the physical and chemical properties of muscle and quality must be established. This information can then be used to devise objective methods for measuring the degree of tenderness, juiciness, and flavor in meat cuts.

The dominant method of merchandising meat in retail stores today is through the use of self-service display cases. Therefore, quality and appearance of the meat is of primary importance and research on maintaining meat quality and shelf life is a necessity for the success of this type of merchandising. Lighting conditions required to evaluate meat quality need to be defined so that the meat quality attributes can be properly assessed.

The maintenance of desirable meat quality during various transport techniques and the determination and evaluation of the various methods of shipping fresh meats to European markets are primary research needs if we are to expand our market for fresh meats.

#### USDA PROGRAM

The Department has a continuing program involving chemists and food technologists in basic and applied research on quality maintenance, and development of objective methods for quality evaluation of meat. This work is conducted at Beltsville, Maryland, partly in cooperation with the Animal Husbandry Research Division, ARS, and the Livestock Division, C&MS, and partly by contract with the Universities of Wisconsin and Missouri.

The Federal scientific effort devoted to research in this area exclusive of contract research totals 6 professionals divided as follows: quality evaluation, 5.0 and quality maintenance, 1.0.

A research grant to the Research Center of the Meat Industry, Helsinki, Finland, provides for a study on the effects of carbon dioxide or nitrogen on chemical and physical properties of refrigerated meats. Its duration is 4 years, 1963-1967, and involves P.L. 480 funds with a \$44,453.40 equivalent in Finnmarks.

## PROGRAM OF STATE AGRICULTURAL EXPERIMENT STATIONS

Research directed to increasing our understanding of the market quality of meat has been a continuing part of the State stations' research program. Both basic and applied research are involved.

Market quality research on meats begins with study of the influence of breeding, feeding, and management treatments with cattle, sheep, and swine on the carcass and meat quality characteristics. The objective is to determine the relationships of live animal and management factors to ultimate eating quality. Such live animal traits as birth weight, rate of gain, efficiency of gain by sire groups, body measurements such as depth and length of body, type, market weight, and grade are related to carcass traits such as loin eye area, muscling characteristics, amount and distribution of fat, yield of wholesale cuts, chemical composition and carcass value in an effort to define animal traits which influence carcass and meat quality.

Other research involves investigation of various pre-slaughter treatments on the carcass quality, organoleptic characteristics and market value of the meat. Special attention is given to tenderness of meats and the fundamental causes of toughness or tenderness in meats. Certain post-mortem factors including aging exert profound effects on meat quality and considerable effort is devoted to attempts to gain a better understanding of their effects.

Almost all of the studies involve a certain amount of work on methods since methodology is of vital importance in the study of quality factors. Development of objective criteria for evaluation of meat quality is a continuing goal and new and improved methods of defining the quality of meat cuts are constantly sought.

Further along the route to the consumer, concern arises as to the effects of processing and storage treatments on the quality of meat. The influence of maturity, marbling, methods of aging and processing and storage, packaging and distribution are all studied for possible effects on ultimate quality. Microbial quality, distribution of muscle proteins, and lipids, morphological features, amount of connective tissue, and cooking treatment are other factors considered in attempting to establish the total quality characteristics of meat. Finally, the relationships of raw and cooked meat quality to consumer preference are determined. These are in turn related to the carcass quality and market value of the live animal.

A total of approximately 17.7 professional man years are devoted to market quality research on meats.



## REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Objective measurement and evaluation of quality

1. European Fresh Beef Shipments. This work in cooperation with the Transportation and Facilities Research Division, ARS, is to study techniques for shipping fresh beef to European Markets. Preliminary studies were conducted on: (1) refrigerated van containers, (2) American-flag refrigerated holds, (3) foreign-flag refrigerated holds and (4) banana boats. Various methods of stowing fresh beef in refrigerated ship's holds showed beef hung on racks constructed of wood uprights and steel pipes with one layer of beef quarters on the floor used more of the available load space, and delivered the fresh beef quarters in excellent condition. These studies indicate that it is possible to ship American fresh beef to European markets in excellent condition and at relatively low transportation costs.

(MQ 3-34)

2. Lighting Requirements for Evaluation of Meat Quality. This study is designed to survey and assess the present lighting conditions under which meat is graded. Preliminary data indicate considerable variation in the light intensities within a beef cooler where a meat grader determines the quality grade of beef.

(MQ 3-59)

3. Relationship of Marbling to the Palatability of Beef. Results to date indicate the phospholipid content of five different muscles varies from approximately 0.7% to 1.25% depending on the muscle. The variation in triglyceride content is considerably wider ranging from 1%-10% of muscle weight. Cholesterol content is roughly 0.5% of the total lipid extract and is related to the triglyceride content rather than to phospholipid content. The degree of marbling plus this type of information may provide a more objective method for quality evaluation.

(MQ 3-60)

4. Flavor Studies to Provide a Basis for More Objective Measurements of Meat Palatability. Both precursor systems and the compounds responsible for meat flavor are being studied in beef and lamb. A fraction has been isolated from untreated lamb fat that has a characteristic lamb aroma. The intramuscular fat is being studied to see if this lamb flavor resides solely in the depot fat or is characteristic of all lipids in lamb.

The differences in the fatty acid composition of lipids results in the formation of different types of carbonyl compounds when meat is cooked and these compounds are potent flavor contributors.

(MQ 3-61)

5. Objective Methods for Measuring Maturity. The proteolytic activity at pH 3.8 was determined in breast and thigh muscle of six sets of broilers ages 4, 6, 8, 10, 12, and 14 weeks. The results show that in young, rapidly growing birds proteolytic activity is high and decreases with maturity. The direct relationship found suggests that proteolytic activity is related or involved in protein turnover and/or synthesis. This research approach is being investigated as a means of determining maturity in meat animals. Disc electrophoresis of water soluble proteins extracted from chicken muscle of 4- and 6-week old birds gave protein patterns that were qualitatively and quantitatively different from patterns obtained from birds 8-to 14-weeks old; this study has been extended to beef muscle. These differences in patterns may provide a method for measuring maturity.

(MQ 3-62)

B. Quality maintenance in handling and packaging

1. Shelf-Life of Prepackaged Meats. A manual containing recommendations for temperature, humidity, sanitation, and handling procedures for fresh meats, prepared under USDA research contract has been published by the University of Missouri Cooperative Extension Service. Steps that a retailer can take to increase the display shelf-life of meat are presented in the manual. Laboratory tests showed that meat displayed at 30°F had significantly longer shelf-life, less weight loss, lower bacterial counts, and more desirable color than meats stored at 34°F or 38°F. Also prepackaged meat samples held in darkness prior to display had significantly better color and desirability scores than those samples displayed under 120 c.p. of light. Little differences were found in the myoglobin percent, however, in most instances the percent metmyoglobin was significantly less from meat samples held in darkness. There were no significant differences in bacterial counts between the two light treatments.

(MQ 2-75)

C. Quality maintenance during transportation

1. Effect of Atmospheres of Carbon Dioxide and Nitrogen on Properties of Refrigerated Meats. Investigations on meat samples stored in 10% and 20% CO<sub>2</sub> were reported. They showed that control meat samples had slime in 12 days and a spoiled odor at 19 days whereas slime was produced in 33 days under 10% CO<sub>2</sub> and in 40 days under 20% CO<sub>2</sub>. Color of the control samples had greater metmyoglobin than those stored under atmospheres of 10% or 20% CO<sub>2</sub>. Other levels of CO<sub>2</sub> and N<sub>2</sub> atmospheres are scheduled for future studies.

(E8-AMS-5(a))

## PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Quality Maintenance in Handling and Packaging

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(MQ 2-75)

Quality Maintenance During Transportation

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(MQ 3-34)



**LIVESTOCK, MEAT, AND WOOL -  
MARKETING FACILITIES, EQUIPMENT AND METHODS  
Transportation and Facilities Research Division, ARS**

Problem. Many of the livestock, meat, and wool marketing, slaughter, and warehouse facilities occupied today are obsolete and the work methods that can be used in such facilities are antiquated. As a consequence, labor costs are excessive and they are increasing. Many firms still are occupying facilities designed primarily for handling rail receipts and rail shipments even though the majority of these products today are moved by motor-truck. This situation also adds to handling costs. Numerous firms are occupying "makeshift" facilities which were designed for other uses or for work methods and operations of a bygone era when labor costs were low. Changes in transportation systems, population growths and shifts, and advancements in technology also have brought about changes in the types of facilities--such as livestock auction markets, commercial feedlots, and hotel supply houses. Most private firms handling livestock, meat, and wool lack the technological and engineering skills necessary to plan and develop suitable facility layouts and designs and to select the types of equipment needed. Therefore, engineering and related research is needed to provide guidelines for industry to increase efficiency; including the designing of improved plant layouts, which will provide proper arrangement of work areas to minimize travel distances and excessive handling and the development of work methods that will permit use of mechanized and automated equipment rather than the relatively high-cost manual methods now used in many plants.

#### USDA PROGRAM

The Department has a continuing long-term marketing research program involving agricultural and industrial engineers, agricultural economists, and meat scientists engaged in both basic and applied research to develop new and improved methods, equipment, processes, and facilities for livestock markets, meatpackers and wholesalers, and wool warehousemen. Livestock market research is carried on at Columbia, Mo., in cooperation with the Missouri Agricultural Experiment Station. Part of this work also is in cooperation with the Central Missouri Livestock Auction, Mexico, Mo. Work on the automatic driving and penning system is under a contract with the American Research and Manufacturing Corporation, Rockville, Md. Research on livestock slaughtering and on meatpacking and wholesaling is headquartered at Stillwater, Okla., and is cooperative with the Oklahoma Agricultural Experiment Station. Wool warehouse research will be conducted at the Columbia, Mo., field location.

The Federal effort devoted to research in this area totals 5.8 professional man-years; 2.6 man-years (0.5 man-year intramural and 2.1 man-year extramural) on livestock; 2.5 man-years (2.0 man-years intramural and 0.5 man-year

extramural) on meat; and 0.7 man-year on program leadership. No research is currently being done on wool warehouses.

## REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

### A. Automation of Sales Operations on Livestock Markets

At Columbia, Mo., the construction of prototype electrically-operated gates for use at the entrance and exit of the sales ring of livestock auction markets was completed by the Missouri Agricultural Experiment Station under a research cooperative agreement. The gates are powered by  $\frac{1}{2}$ -hp. electric motors and use an electrically-operated latching system. The first of the prototype gate assemblies was laboratory tested through 53,000 cycles; the latch assembly for the gate was tested through 200,000 cycles.

At the end of the report year, modifications were being made in the gate structure to reduce its weight. Tests under actual market conditions will be conducted during fiscal 1966 on the Central Missouri Livestock Auction, Mexico, Mo.

### B. Determining Behavioral Patterns of Livestock

The results of this research, conducted under a contract with American Research and Manufacturing Corporation, Rockville, Md., were covered last year. The purpose of the research was to establish behavioral patterns of cattle, hogs, and sheep under environmental conditions existing on stockyards and auction markets to determine the feasibility of driving and penning livestock automatically. The findings of this research provided the information needed to negotiate a contract to design, construct, and test a mechanical driving and penning system for livestock markets.

The contractor's report covering the research results was checked, organized, and a revised draft for Department publication was prepared. At the end of the report year the manuscript was in the hands of the Division editor.

### C. Development of an Automatic Driving and Penning System for Livestock Markets

Under a contract with American Research and Manufacturing Corporation, Rockville, Md., a prototype mechanical driving and penning device for livestock markets was constructed. The pen gates are opened and closed by a solenoid operated latch and a reversible electric motor. The holding pen gate at the head of the pen-back alley pivots on a horizontal axis located about nine feet above the floor. The latch on this gate is controlled by a solenoid and is pivoted with solenoids.

The driver is electro-mechanical and operates on a trolley rail. It is equipped with electrically charged bars which provide the stimuli for driving and penning livestock. It is capable of driving livestock to any selected pen on the pen-back alley, and return to the starting position within a specific programmed time limit.

The device was designed, constructed, and laboratory tested at Rockville, Md., during the past year. Following acceptance of the report on the laboratory tests, the unit was disassembled and shipped to Mexico, Mo., for installation and testing under actual operating conditions on the Central Missouri Livestock Auction. At the end of the report year the contractor was making adjustments in the electrical controls which had delayed the starting of the tests under market conditions.

#### D. Layouts and Work Methods for Wool Warehouses

At Hyattsville, Md., a manuscript entitled "Layouts and Work Methods for Wool Warehouses" was completed and published in May 1965.

#### E. Layouts and Work Methods for Hog Slaughtering Plants

At Stillwater, Okla., a draft of a report entitled "Hog Slaughtering and Dressing Systems" was completed. The report evaluates the costs of operating three different types of hog slaughtering and dressing systems and compares the relative efficiency of various work methods and types of equipment used in performing the required operations. The systems reported on include the powered bleeding rail, gravity bleeding rail, and chain bleeding conveyor types of operations. Results of the study show that the powered bleeding rail system was the least costly and the gravity bleeding rail system the most costly. The powered bleeding rail system can be operated at an annual savings of approximately \$8,736 as compared with the gravity bleeding rail system. The chain bleeding conveyor system would permit an \$8,658 annual saving as compared with the gravity bleeding rail system. Efficient layouts for each of the three systems were developed from the basic data accumulated and are presented in the report.

At the end of the year the report was being reviewed to incorporate editorial suggestions and comments.

#### F. Layouts and Work Methods for Hotel and Restaurant Meat Supply Houses

At Stillwater, Okla., a manuscript entitled "Hotel and Restaurant Meat Purveyors - Custom Service Houses - Improved Methods and Facilities" was completed and submitted for publication. A revised draft of a second manuscript entitled "Hotel and Restaurant Meat Purveyors - Frozen Portion Control Houses - Improved Methods and Facilities" was also completed. The results of this research were covered last year.



#### G. Handling and Processing "Hot" Pork Carcasses

At Stillwater, Okla., under a research cooperative agreement, the Oklahoma Agricultural Experiment Station continued the study to determine the feasibility of fabricating, curing, smoking, boning, and chilling pork cuts from the "hot" carcass. Sixty pork carcasses have been processed and the research data analyzed. Results of data obtained this year shows that:

1. Curing brine can be pumped into the "hot" ham or belly and items placed directly into the smokehouse.
2. Processing hams from kill to finished product can be accomplished in 15 hours by using this new technique.
3. Tenderness, yield and moisture content showed no significant differences from conventionally processed hams.
4. "Hot" processed bacon can be formed with no significant differences in width and length from "cold" processed bacon if the hot belly is stretched or held in its original shape during the time it is in the smokehouse.

Some of the data gathered from processing the 60 pork carcasses still remains to be interpreted. At the end of the report year plans were under-way to process an additional 20 hogs to obtain data for microbiological, color retention, and heat transfer analyses.

#### H. Layouts and Work Methods for Small Inedible Rendering Plants

At Stillwater, Okla., field work was initiated to improve work methods, equipment and facilities for small inedible animal fat renderers. The applicability of larger continuous type rendering processes to smaller volume plants will be an area of consideration for evaluating batch-type operations. Work has not progressed sufficiently to report research results on this project.

#### I. Layouts and Work Methods for Beef and Veal Boning Lines

At Stillwater, Okla., field studies were initiated to develop more efficient work methods, equipment and layouts for beef and veal boning lines. Studies will include both mechanized and manual handling of meat, bones and fat. Studies will determine the volume breakpoints for efficient utilization of various equipment types and systems.

Work on this project has not progressed to the stage where significant findings can be summarized.

## PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE PROGRAMS

Layouts and Work Methods for Wool Warehouses

Webb, Tarvin F. 1965. Layouts and Work Methods for Wool Warehouses. Marketing Research Report No. 667. 36 pp.

Handling and Processing "Hot" Pork Carcasses

Hammons, Donald R., and Webb, Tarvin F. 1965. Fast Processing for Smoked Ham. Agricultural Marketing, Vol. 10, No. 4, April 1965.

## ECONOMICS OF MARKETING

### Marketing Economics Division, ERS

Problem: Economic research in agricultural marketing revolves around the problems of increasing efficiency in the processing and distribution system and providing a foundation for orderly adjustments to changes inside and outside of agriculture. Marketing must be looked upon as a dynamic and changing process. The capacity to adjust to and cope with the dynamics of modern marketing is required increasingly of producers and distributors of farm products. Demands of a more knowledgeable and sophisticated consuming public are adding to the pressures for an even more rapid escalation of developments and changes within the marketing system. Changes in institutions and redirection of public policies and programs are modifying the economic environment in which marketing firms must perform and operate. Because of rapid changes and increasing complexities associated with a dynamic marketing system, it is necessary that a continuous program of research be conducted in marketing--a program aimed at keeping producers and marketing firms abreast of the flow of events and providing information necessary to them in making proper and orderly adjustments to change.

Of increasing economic concern is the problem of how to improve and strengthen markets for farm products in face of a continuing rise in production, higher distribution costs, and competition from nonagricultural products. The problem of increasing demand for farm products to meet rising productivity has become progressively more pronounced in the last decade. Interest in the development of markets has mounted as larger and larger financial outlays become necessary for price-support operation and maintenance of reasonable levels of farm income.

### USDA AND COOPERATIVE PROGRAMS

The Department has a continuing long-range program of economic research directed in two major areas: (1) Organization and performance of markets and (2) development of markets. Research on organization and performance is designed to increase the efficiency of marketing and assist producers and marketing agencies in adapting to a changing environment. Research is conducted on a wide range of functional and commodity problems that arise in moving farm products from producers to consumers. The program involves both basic and applied research and is primarily oriented to problems of national and regional scope. Field studies are often conducted jointly with State agricultural experiment stations, with processors and distributors of agricultural products, transportation agencies, and agriculturally-oriented trade groups. Producer groups and trade organizations have, with increasing frequency, made financial contributions to the Division research efforts. Many staff members are working closely with the staff of the



National Commission on Food Marketing in a consultative role and in research studies on price spreads and market structure. These studies are partly financed by the Commission. Of this research effort, approximately 26 professional man-years were devoted to animal-poultry and products, of which 8.5 were for dairy; 8.0 for poultry; 8.5 for livestock; and 1.6 for wool.

Research on development of markets consists of both basic and applied research on agricultural commodities which includes the development of general principles in advertising and promotion, appraisal of public food distribution programs, and evaluation of the commercial feasibility and market potential for new and improved products. Of this research effort 8.5 professional man-years were devoted to animal-poultry and products.

#### PROGRAM OF STATE EXPERIMENT STATIONS

##### A. Market Institutions and Market Power

Eighteen projects at as many stations are concerned with commercial egg marketing, 11 of which contributed to the NCM-31 regional project on coordinated egg production and marketing programs, and 7 contributed to NEM-21 which is evaluating the economic feasibility of alternative egg marketing systems in the Northeast. A total of 26.5 professional man-years at State experiment stations is involved--12.2 are for broilers and 14.3 are for eggs.

The State experiment stations are conducting considerable research at the regional and State level in order to gain a clearer understanding of changes in the livestock marketing system. Some of the questions being studied are: (1) What proportion of the livestock is now being sold direct, through auctions, and through terminal markets? (2) what is causing the trend away from terminal markets? (3) what is the geographical structure of the livestock marketing and processing industry and what is causing it to change? (4) what is happening to the old established meatpacking plants? (5) what kind of new firms are coming in? (6) are the types and location of marketing services and facilities changing? (7) what is the impact of the chain stores on the meat processing industry? and (8) what impact is new processing, transportation, and communication technology having on the industry's structure?

With all the changes taking place in livestock and meat marketing, particularly the trend toward concentration of volume into the hands of national food chains, close attention is being paid to changes in market structure, conduct, and performance and to what might be done to improve the competitive tenor of the markets. A small amount of research is evaluating the adequacy of market information.

Current research on wool is directed toward appraising existing marketing practices and evaluating alternative channels of sale. The number of professional man-years involved is 24.

Stations in the southern, north central, and western regions are analyzing changes in the processing, manufacturing, and distribution of dairy products which are associated with changes in demand, supply, technological, and institutional factors; and to relate these changes to the structure, behavior, and performance of markets. Public policies, which facilitate adjustments within the various regional dairy industries, are also investigated. Additional studies deal with the effects of bulk-milk handling on the structure and efficiency of milk assembly; the degree of consolidation necessary for successful cheese, butter, and milk powder plant operations and the nature and type of competition associated with structural changes in the dairy industry. The station support of this research amounts to 22.2 professional man-years.

#### B. Prices, Margins and Costs

Prices and marketing margins are continually being examined in light of changes in production, processing, storing, transporting, and distributing agricultural products. Historically, retail prices have increased relatively more than farm prices which is a reflection of increased marketing services of many products. There is a high degree of public and legislative interest in this research being conducted by the State stations.

Sixteen projects on marketing poultry and poultry products are in operation at 15 stations. Ten studies on eggs are concerned with adjustments in the egg-products industries, investigating trade practices costs and egg quality; analyzing country point egg pricing systems; marketing an economic analysis of alternate systems of materials handling for egg packing plants; and developing an analytical model for a least-cost table egg marketing complex. Five studies on poultry meat marketing are concerned with techniques for increasing demand for locally grown broilers, effect of marketing organization on broiler marketing costs, shifts in supply and demand for broilers and their effects on future marketing resource requirements, prospective demand for broiler meat in the South, and efficient processing and utilization of chicken meat. One study is directed at improving the techniques for predicting prices and marketing of poultry. A total of 9.4 professional man-years is assigned to poultry and poultry products research including 4.9 on eggs, dealing with prices, margins, and costs.

A variety of studies are underway on livestock prices including prices received by farmers for livestock sold through different marketing channels, of trends in seasonal price patterns, of prices in particular markets, of supply-demand relationships, of the effect of meat promotion on prices, of factors affecting feeder cattle and feeder pig prices, and of consumption



trends and potentials. These studies in some instances are being synthesized to project future price and market conditions. Some studies are being made of the adequacy of livestock grades and standards in estimating meat yield and quality. A number of studies are in process on marketing margins on meats, including ways of reducing marketing margins through improving the physical efficiency of marketing facilities and processing plants. One project on wool seeks to reduce the handling cost on wool by finding improved baling and packaging methods. Total man-years in this research area amount to 30.3.

Dairy marketing research by the State stations is concerned with the supply, demand, price, and utilization of dairy products. Studies estimate the demand and supply functions for Grade A milk and equilibrium price levels; investigate marketing and merchandising methods that influence the demand for and sales of milk, and analyze the efficiency of marketing operations and methods. Other studies are exploring equilibrium prices and product flows in the Northeast and price differentials and product flows between the northeast and the north central regions. Studies also are underway to determine the acceptability of product innovations such as "fortified" skim milk; the demand for and potential supply of butter of various flavors and quality; the economic evaluation of pricing plans and payment systems for milk, and the feasibility of pricing plans based on the various components of milk.

Another segment of dairy marketing research deals with costs, margins, and efficiency of operations. A cooperative regional project in the Northeast determines the effects of economic, technological, and institutional conditions upon the costs and efficiency of milk assembly, processing, and distribution. Other studies deal with the production and marketing of semi-perishable dairy products; the economic effects of bulk-milk handling; and the economic efficiency of cheese and dry milk production. The station support of this research amounts to 28.9 professional man-years.

### C. Location and Interregional Competition

Changes in the technology of producing and processing agricultural products has much impact on the comparative advantage of one producing area over another. Changes in transportation rates and costs also affect the competitive position of some areas more than others. These changes and their effects on the location and interregional competition are being studied by the State stations.

Three stations have four projects on poultry and poultry products marketing. One study deals intensively with factors and potentials for the competitive position of the Delmarva broiler industry, its economic advantages and disadvantages, market potential and adjustments needed to realize them. The other studies are concerned with spatial equilibrium models for eggs and for



broilers, each with specific area differentiations showing supply, demand, equilibrium price, and transfer costs. Another study is determining regional supply, consumption, price, and shipping patterns of poultry products, including impacts of potential foreign markets. It involves a total of 2.9 professional man-years.

Because of the highly dynamic nature of the livestock and meat marketing system there is considerable research interest in discovering the factors that determine the optimum location for livestock marketing and processing facilities. This interest extends into an analysis of the comparative advantage of competing areas in fattening cattle, taking into account access to feeder cattle, feed grain supplies and prices, and nearness to consuming centers. Changes in meat processing technology and retail organization have tremendous implications as to where meat processing facilities can best be located. Changes in transportation technology and rates and in highway development also affect optimum location. Total man-years in this effort are 15.5.

#### D. Merchandising and Promotion

Research at State agricultural experiment stations in the area of merchandising and promotion is problem oriented although conducted on specific commodities.

Six stations have projects on poultry and poultry products. Two are intensive studies of product development and market evaluation of new products from eggs and poultry meat. One is an analysis of the effects of various methods of processing, packaging, merchandising, consumer education and advertising on sales. Another is aimed at developing markets and merchandising practices for inedible byproducts of the poultry industry.

Specific commodity studies include: Conditions and practices affecting marketing costs and consumer acceptance of heavy young chickens; potential effects of new and improved methods of merchandising turkey meat upon purchases by consumers; and effect of labeling and providing information on nutrition of eggs on the price structure and its relation to quality market grades. A total of 7.3 professional man-years at the State experiment stations is allotted to this research dealing with merchandising and promotion.

Dairy merchandising studies underway at the experiment stations are designed to discover and test methods and techniques which may increase the sale of dairy products and increase the efficiency of their distribution. Another project analyzes past and present utilization patterns for milk and dairy products and determines the effects of factors influencing these patterns. The stations' efforts devoted to this area of research amount to .5 professional man-year.

## E. Distribution Programs and Market Outlets

Two projects are underway at the experiment stations concerning the milk and school lunch programs. One deals with the pricing and transfer of milk quotas and the other with the effects of school lunch programs, surplus disposal programs, and Federal order markets on milk and dairy products' consumption. Research effort devoted to this work amounts to 1.2 professional man-years.

### PROGRESS--USDA AND COOPERATIVE PROGRAMS

#### A. Market Institutions and Market Power

The structure of markets for agricultural products is changing in many dimensions and the market power of buyers and sellers and practices of marketing firms are shifting in response. All these changes have significant impacts on farmers, consumers, and marketing agencies. The Marketing Economics Division conducts studies on many phases of this changing market.

Data from a study on price behavior in retail food stores of different ownership types show practices at this level related to price levels and changes. For example, a preliminary analysis of prices of whole broilers on each Tuesday and Friday during a period of a year in a sample of stores in two North Carolina cities showed important differences by day of week, and over longer periods of time. In general, prices in affiliated stores averaged lowest throughout this year while the unaffiliated independents maintained the highest level. For stores of the same ownership types, the daily price range was smallest among chain outlets and largest for unaffiliated independents.

When prices were averaged over a period of a month, chain and affiliate prices tended to rise and fall with farm prices in North Carolina and prices paid by retailers in Atlanta. On the other hand, the wide fluctuations in retail prices between Tuesday and Friday in many of these stores were apparently unrelated to changes in prevailing farm and wholesale prices of fryers as shown by the Federal-State Market News Service. Owners of independent stores appeared to determine prices without much regard to current prices to producers or to those in other stores.

In most agricultural industries, the numbers of plants and companies have been declining in the postwar period. For example, the number of fluid milk plants has been declining for at least 75 years and most other types of dairy plants for 30 or 40 years. Technological and economic changes make it increasingly difficult for the small plant to compete with larger plants. In the livestock and poultry slaughter industries, however, the number of plants has been increasing. New poultry slaughter plants were built to handle the increased volume of broilers. The shift of livestock slaughter plants from terminal cities to interior points along with a shift from multi-product and multi-specie plants to specialized slaughter plants has helped to increase the number of plants.



During this same period, concentration--as measured by the market share of the four largest companies--has declined or remained steady in most of these industries. The proportion of Federally-inspected livestock slaughter accounted for by the four largest firms declined from 51 percent in 1950 to 35 percent in 1962. In this same period, concentration increased fairly rapidly in small fluid milk markets, changed very little in middle-size markets, and declined slightly in the largest markets.

The nature of competition in the food industry is changing markedly. Chains and affiliated retailers are increasingly adopting private labels--their own brands--of products, where these were scarce only a few years ago. In a recent study in the Midwest, 58 percent of the chains had private label brands of fluid milk; 80 percent, ice cream and bulk natural cheese; 75 percent, butter; and 52 percent, nonfat dry milk. Usually the private label brands were priced below packer brands. Eighty-two percent of the stores priced private label half-gallons of fluid milk lower than processor brands.

Integration is increasing in the turkey industry. More turkeys are being produced under risk-sharing contracts, primarily with feed firms--about 25 percent of the total production--or firms producing turkeys on company-owned or leased farms--approximately 10 to 15 percent of output. Another 20 to 25 percent are produced with credit from banks and other financial institutions and about 45 percent are financed by credit from feed companies and other sources. These categories overlap and are not additive.

The procurement systems used by chains and other large-volume retailers for eggs illustrate the changes taking place in the marketing system. The most popular system is where the eggs move from producers to assembler-distributors directly to warehouses or individual stores of large-volume retailers. The typical system of the 1930's moved eggs through three or more handlers before they reached the retail store.

The base price quotation system has been in use in the egg industry for many years. The reason for its widespread use is that large-volume retailers and other buyers want to have a common denominator in negotiations with suppliers. The main area of negotiation is how much above the base price quotation will be paid for consumer-graded, cartoned eggs. Alternative pricing systems are relatively few and point in the direction of administered pricing in one form or another. The industry seems to prefer free market pricing with all of its shortcomings.

Recent and significant changes in the structure, locations, and practices of the wool trade have resulted in considerable confusion among growers and marketing agencies. The growers and marketing agencies have little information to use as a guide in their efforts to meet changing market requirements. The suddenness and scope of these changes accentuated the need for an evaluation of the adequacy of the domestic wool marketing system. Pertinent 1964 data have been collected from more than 2,800 wool producers, 47



local pool operators and 38 warehousemen throughout the U.S. A preliminary report for each segment is being prepared. It has not been possible to include wool manufacturers in the study as was originally planned. The study is conducted in cooperation with the National Wool Marketing Corporation and the American Farm Bureau Federation.

## B. Prices, Margins, and Costs

In the last decade, prices to consumers for most products of farm origin have risen despite downward trends in farm prices. This widening of farm-retail spreads has brought widespread public concern about the efficiency and performance of the marketing system which culminated in the establishment of a National Commission on Food Marketing.

The Division staff in recent months has answered an unusually large number of requests for information about changes in prices and spreads. In mid-1965, higher food prices were the principal force behind a rising consumer price index. Higher prices for livestock and some fruits and vegetables were responsible for most of the increase; the total farm-retail spread for a market basket of food products was generally unchanged.

In general, farm-retail spreads have widened because of the rising prices of labor and other items that marketing firms buy. However, it is increasingly evident that there is less correlation between short-run changes and farm-retail spreads for individual products and the actual costs of performing marketing services. This results from processing and retailing firms becoming more involved with a multiple-product price concept as these firms extend the number and variety of products and operations. As farmers become more specialized, farmers and their representatives have an increasing interest in prices, returns, and their share of the retail price for a single commodity. Conversely, the processor and especially the retailer have less interest in any single product and more interest in overall gross and net returns on an aggregate or total market basket of foods (often including many nonfarm products).

Special attention in recent months was focused on the price movements of beef, wheat, cotton, and some fresh fruits and vegetables. Research findings on price lags and the effect of retailers' policies on specializing proved especially helpful in explaining price movements for live cattle and beef. Questions on wheat and cotton centered on the impacts of proposed Government programs on prices to consumers. Reduced supplies of potatoes and lettuce in early 1965 brought sharply higher farm and retail prices.

The farm food market basket statistics were revised to make use of the latest data on consumer expenditures in weighting individual foods. Also, changes were made in the products in the market basket to reflect changes in consumer purchases during the last decade. Through the revision, a more accurate measure of the spread between retail and farm prices was obtained, providing a basis for better assessment of market performance. Studies are underway with industry groups to further improve price-spread series for beef, bread, and other products.

The interrelationships between market organization and market efficiency are seen in a study of efficiency in managing the total milk supply in fluid milk markets. In one case, a central producer's cooperative could supply milk to the Pittsburgh handlers at a lower cost and with less reserve milk than would be needed if each handler were securing his milk directly from producers. A central source could provide a uniform weekly supply with only 60 percent as much reserve as that needed by individual handlers.

The operating efficiency of a firm can be increased markedly by providing better information to its management and thus contribute to greater efficiency in the operation of the entire marketing system. Case studies in fluid milk operations indicated that with no increase in the accounting staff, substantial amounts of added information could be provided to management. In the case studies, costs declined and profits increased during the study period, indicating that additional information can be used by management to improve operations. Detailed analysis of the profitability of individual milk routes indicated that there is more earnings potential in focusing attention on several variables which contribute to route performance than in attempting only to control earnings.

Operating costs for 70 firms distributing fresh milk increased to \$4.71 per 100 pounds of milk and cream processed in 1964 (average for first half of year) from \$4.35 in 1954. Two-thirds of this increase resulted from rises in salaries, wages, and commissions--costs accounting for about half of the total operating costs for each year. Several other expense items, however, increased by a larger percentage than payroll cost. Raw material costs of these firms decreased to \$5.79 per 100 pounds of milk and cream processed in 1964 from \$5.90 in 1954, partially offsetting the increase in operating costs. Cost increased more than receipts, however, so net profits declined from 44 cents to 26 cents per 100 pounds of milk and cream processed.

The cost of assembling live turkey increases as firm size increases, and decreases as the production density increases. Costs for a small firm range from .2 cents per pound for high density to about .35 cents per pound for low density. Costs of a firm handling 70 million pounds range from .25 cents per pound for high density to .5 cents per pound for low density. Most of the economies of scale in turkey poult hatcheries are realized when a hatchery sets about 5 million eggs per year. In feed mills, the cost per ton of feed in large mills is about half that of small mills. Substantial economies of scale exist in the manufacturing of further processed poultry products.

One of the fundamental weaknesses of the present system of marketing domestic wool is the lack of adequate classification and market information services. Research was designed to determine the effects of a wool classification and market information service on prices and to study providing such a service. Results indicate that a wool classification service would generally increase prices to producers of higher quality wool, reduce prices to those of lower quality, but would not significantly affect the national



average price. Some practical difficulties would need to be overcome before a classification service would be feasible. Another phase of the analysis indicates a need for improving the relationship between central market quotations and local prices in the Western States. Results suggest that to the extent wools in particular areas tend to be similar, reporting central market quotations more specifically for these areas will assist in compensating for quality factors being considered by local buyers in addition to fiber fineness and staple length. Average market differences ranged from 6 cents below Boston quotations in North and South Dakota to 10.5 cents above in eastern New Mexico. Additional sources of information should be developed to better relate price quotations to local market conditions. Utilization of USDA's Consumer and Marketing Service livestock reporters for this purpose was tested and appears feasible. This study was conducted in cooperation with the New Mexico State University and the Consumer and Marketing Service.

### C. Location and Interregional Competition

As farm production shifts from one area to another, marketing firms must adjust to the new environment. New firms may be called for in one area while those in other areas must seek other opportunities. Changing transportation rates put one area at a competitive disadvantage compared to others both in production and marketing. Marketing economic studies emphasize the changes taking place and the scope of adjustments needed to meet them.

The organization and operation of markets for bulk milk that moves long distances tend to be loosely coordinated. A study of the buyers and sellers indicated that much of the business is on a spot or seasonal basis with individual firms making decisions on each purchase. Only about one-fourth of the buyers interviewed bought milk from outside sources on a regular basis. Most buyers indicated that a shortage of local supplies was the reason for purchasing outside milk and they preferred the flexibility afforded by outside purchasing. Because of the conditions under which such supplies were needed, purchases were made by shopping around among known sellers of "spot" milk.

Price information on dressed beef available to users in the South reflects prices from nonsouthern markets and does not appear to be adequate for decision making by meat-handling and livestock-producing firms. Packers, retailers, and jobbers in Texas and Oklahoma indicated a fairly strong preference for a dressed meat price reporting service in the Southwest which would reflect supply and demand conditions on heavier beef in Texas and Oklahoma and which would provide price information on lighter type beef carcasses, which is not now available from any source.



#### D. Products and Services

Emphasis is being placed in the current program on ways of maintaining agricultural products' shares of the market--particularly where declines in per-person consumption has occurred. In specific commodity areas, this requires a reliable means of identifying the salient consumer wants or needs and then providing researchers with the information so they can channel the research and development programs to maximize product success and consumer satisfaction. Research of this nature has been undertaken in several important commodity areas.

Changes in consumers' purchasing habits combined with competition from other products are significant factors behind a general downward pattern in per-capita consumption of fluid milk products during the past decade. This decline coupled with increased farm output of fluid milk has created serious problems for the entire dairy industry.

Work is being done on low-fat (2 percent) milk, modified milk (improved flavor through increased total solids), and on sterile milk concentrates. The research is based on the proposition that if the optimum dairy product beverage mix can be determined to meet the continuing changing needs of consumers triggered by health, dietary, and related factors, such a determination should maximize consumption and perhaps halt the decline in milk consumption. The low-fat milk study indicated that this product brings some new users of fluid milk into the market place. However, total fluid milk sales do not appear to be measurably increased by sales of this product. The product has been chiefly responsible for offsetting the sharp drop in fluid whole milk consumption.

Serious problems of a similar nature currently confront the hides and leather industry. The problem is not only of better meeting market requirements but the competition of synthetic leather. We are studying the economics of modifying hide trims so as to better meet competition by providing shoe-makers with hide leather products which most closely meet their raw material needs. Theoretically, removal of less desirable portions of a hide would provide a more uniform product of higher quality and enable hides to compete more favorably with substitutes. Findings indicate that the removal of bellies and heads from a hide (30 percent) at the packinghouse, prior to curing, improves the quality of leather and increases economic returns for all segments of the industry. An analysis also was made to determine economic possibilities for using such hide trim. One possibility indicates that fresh hide trimmings have a value of about 2 cents a pound if converted into edible collagen (sausage casings) or rendered into feed and oil by a new process. Currently, packers lose about 2 cents a pound on hide trim. Export markets were also considered for possible new marketing opportunities.

Another special area receiving increased emphasis is cereal grains and fibers. The need for market potentials research is vital because fiber and grain products are especially vulnerable to market substitution. The emphasis in recent years has been to depict the precise nature of the technical and economic competition facing these agricultural materials so as to provide guidelines on the cost-price relationship and performance characteristics that are needed to resist further market erosion.

On the fiber side, the research approach also includes evaluation of the sales potential of new properties as putting stretch in cotton and eliminating shrink from wool. A study completed in the past year pointed out that machine launderability given to wool fabrics will enhance wool consumption in markets that consumed 131 million pounds of wool, a market now subject to substitution by easy-care synthetics. Similarly, research in the planning stage on cotton will explore the extent to which new cotton finishes and cotton products have expanded particular markets for cotton and how well these new developments are meeting new market requirements.

In addition to specific studies of new product potentials, market potential research includes studies of a broader and more basic nature. Such research is based on the premise that successful physical research on agricultural products has to be built on a solid foundation of objective market requirement so that products of agricultural origin can survive in the years ahead. These studies of a broad cross-commodity nature seek to determine in specific terms whether agricultural commodities are meeting market needs and if not, what research can be done to meet these needs. Additional research goals for these broad studies are to: (1) Provide market intelligence to pinpoint opportunities for physical research; and (2) depict the economic environment and conditions that these research developments will have to meet for survival.

The importance of innovation in the economy in terms of economic growth is obvious. However, the high rate of new product failures adding to marketing costs, which result in increased prices to consumers or decreased prices to farmers, stresses the need for reliable indicators of new product success. A first step in this direction has been taken by market potential researchers who have recently measured some of the factors associated with sales of convenience foods. Results of this research provide an equation which may be of use in predicting success of new products. Another aspect of minimizing new product failures has been the use of the microtesting or small-scale intensive approach in "as early as possible" stage in the development of new food products in the Department's utilization laboratories. Developmental work of this nature has been carried on in the past reporting year on dehydrated pumpkin powder, frozen avocado salad (guacamole), and spray dried whole milk powder.



Market testing of new products has resulted in an increasing number of requests from various groups for consultation and cooperative research projects. In addition, there has been a growing number of land-grant universities where food science departments have expanded without commensurate expansion of marketing research services within the university. Because of this, particular emphasis in recent years has been to strengthen working relations with these institutions by means of consultation and cooperative projects. Cooperative work is underway now with Pennsylvania State University (market potentials for maple sirup and maple sirup products), the University of Hawaii (market possibilities for Hawaii farm products), Clemson University (acceptance of modified beverage milk in Southeastern States), and the University of Wisconsin (an evaluation of selected segments of the institutional market for sterilized milk concentrate).

Continuing emphasis is being placed on oilseeds, particularly the soybean in view of the successful work accomplished on the project relating to the use of whole soybeans for feed in making mixed feeds. Information was developed for farmers, livestock and poultry feeders, and feed mill operators so as to allow them to make a decision as to whether or not processing of soybeans in this way was economically feasible under local conditions. Work is being initiated on the market for safflower oil and meal. This research will tie in closely with the physical research program on safflower oil carried on at the Western laboratory.

#### E. Merchandising and Promotion

The current program of research in merchandising and promotion develops and provides information which enables producers and marketers to be more responsive to changes in demand and to undertake merchandising and promotion which will be most effective in maintaining or expanding demand for agricultural commodities. Major emphasis is placed on evaluation of the promotion and merchandising potential for commodities and the development of general principles applicable to this area of marketing activity.

A study of the relative sales response of fluid milk to three levels of promotional investment--normal promotion expenditures of about 5 cents per capita invested annually, normal plus 15 cents per capita annually and normal plus 30 cents per capita annually--shows that significant increases in Class I milk sales of 4.5 and 5.9 percent were attributable to the 15- and 30-cent levels of intensified promotion, respectively. Based on the differential between prices received by producers for Class I and Class II milk, returns to producers of 59.4 percent on the added investment of 15 cents per capita and 13.5 percent on the added investment of 30 cents were shown. Of the levels tested, the investment of 15 cents per capita above normal gave maximum benefits. This study was conducted in cooperation with the American Dairy Association.



In addition to the development of consumer profiles for commodities, studies are being conducted to delineate channels of distribution and availability and appraise current merchandising and promotion practices for farm products. Results of these studies identify problem areas in the distribution system and provide benchmark data for formulating market development and promotion programs by farm groups and marketing firms. Weak and strong areas of distribution are identified as are specific problems hindering distribution and availability in the weak areas. The importance of direct action by organized farm groups in marketing their product is revealed in the results of recently completed research on the promotional activities and practices of farm groups. This study showed that approximately 1,500 farm groups were engaged in conducting market development and sales promotional programs in 1963. These groups spent an estimated \$92 million on domestic programs, and an additional \$5.6 million on foreign market development activities. This is a relatively new field for many groups and they have limited guidelines for planning a promotion program for evaluating its effectiveness.

Analysis of historical data and data generated through controlled experiments is used to test the sales effectiveness of specific retail merchandising practices (in self-service retail outlets) such as methods of display, pricing policies, consumer packages (types, size, color, etc.) point-of-purchase information, the impact of retailer featuring, and product identification. Findings of recent studies reveal that sales of broilers are associated with amount of retailer advertising, display space devoted to the product, and the addition of other methods of offering "cut-up" broilers. Quantification of these relationships permits retailers to implement specific merchandising plans that will be most effective in moving broilers into consumption. This research also identified and quantified factors affecting prices received by producers for live broilers. The major ones are: (1) season; (2) supply of broilers including cold storage handling; (3) terminal market price of pork; and (4) wholesale price of turkeys.

A study of food stores indicates that space in frozen food departments could be used more effectively if management were to assess the costs and movement of individual products in allocating display area. Primarily responsible for the favorable profit position of frozen foods were high gross margins of 25.6 percent of sales and low variable cost of 3.7 percent. Three product groups--drinks, vegetables, and dinners--accounted for over half the sales of frozen food during the experiment. Gross margins, rate of turnover, and returns per square foot of display space showed considerable variation among the 13 product groups in the frozen food department.

In like manner, research conducted and underway point up the complexity of meat merchandising relative to sale effectiveness and the need for management tools not presently available. Fresh poultry and beef products increased over 2.5 times and pork loin 1.4 times normal when each was featured. Since retail labor costs vary considerably among meat products, it would appear that this cost could be reduced if management were to give more attention to labor requirements and product movement in determining product features. The response of individual primal cuts to advertising

and price change is being further analyzed to clarify the effectiveness of sales practices--information essential to management in more effectively and efficiently merchandising of meat.

#### F. Distribution Programs and Market Outlets

How well public distribution programs meet the needs of the people to whom they are directed and their effectiveness in developing stronger markets for farm products are examined by a continuing research effort. Answers to these questions when related to program cost and their effect on farm income provide a sound basis for program administration and for policy decisions and legislative action in formulating and executing public food distribution programs and developing market outlets.

Findings from a study of the market for food in schools indicate more than \$1 billion in food (wholesale value) was used in school lunchrooms during 1962-63. Approximately 80 percent of this food was obtained locally. Comparisons with an earlier study of public schools (1957-58) indicate an expansion in value of foods by 56 percent in 5 years. This gain resulted primarily from more children eating. However, average value of consumption also increased--primarily meats and dairy products. Findings from the market for food in school surveys and related studies concerning availability of school lunches and milk have contributed to the proposed implementation of special Federal assistance under the National School Lunch Program to schools with a high percentage of needy children in attendance and have been used widely in other program evaluations.

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**COOPERATIVE MARKETING**  
**Farmer Cooperative Service**

**Problem:** Farmers continue to increase their use of cooperative marketing.

These cooperative operations are conducted in a marketplace where handling and processing, transportation, and distribution technology is changing rapidly, and market organization and practices are undergoing major changes. Farms themselves have changed. Farmers and their cooperatives need research results that relate to these developments and new conditions to assist them in marketing efficiently. Such research will assist farmers to strengthen their bargaining power, increase marketing efficiency, and meet effectively the quality, quantity, and service needs of today's food and fiber marketplace.

Cooperative marketing is a direct and major way for farmers to get maximum returns from their products. Farmers own and operate cooperatives specifically to increase their income from crops and livestock. Gains are not automatic, however. Cooperatives must plan and actually conduct the specific marketing program and services that will yield best returns for their members. Marketing cooperatives must know what the consumer demands, as reflected in the market. They must be able to estimate the cost of serving the market in different ways. They must understand the possibility of major economies in a well-managed joint sales program, and understand the methods and potentials of bargaining. Management must achieve minimum costs through appropriate organization, good use of existing plant and personnel, and the correct selection and use of new equipment and methods.

**USDA PROGRAM**

The Department conducts a continuing long-range program of basic and applied research and technical assistance on problems of marketing farm products cooperatively. Studies are made on the organization, operation, and role of farmer cooperatives in marketing. While most of the research is done directly with cooperatives, the results are generally of benefit to other marketing firms. The work is centered in Washington, D.C. Many of the studies, however, are done in cooperation with various State experiment stations, extension services, and departments of agriculture.

**Federal professional man-years devoted to research in this area totaled 9.3. Of this number, 3.8 was devoted to dairy, 2.0 to livestock, 3.0 to poultry, and 0.5 to wool.**

Research also is conducted under contract with land-grant colleges, universities, cooperatives, and private research organizations. This report includes work conducted during the present period, or release of results of

work earlier completed, through contract research performed by universities in Iowa, Montana, North Dakota, and West Virginia, and by two private research companies.

#### STATE EXPERIMENT STATION PROGRAM

Most of the commodity marketing research of the agricultural experiment stations is helpful to marketing cooperatives. Some projects, however, deal specifically with cooperative marketing problems, opportunities, and impacts. At the present time 10 States have 12 research projects in cooperative marketing. Their commodity distribution is as follows: grain-2, tobacco-1, fruit and vegetables-1, livestock-2, and cross-commodity-6.

Some projects evaluate the performance and organizational features of cooperatives. Different methods of pooling and their problems are studied so as to develop helpful principles. In the analysis of cooperative operations and in working with directors and managers, efforts are made to identify and solve the many problems that are arising. Particular attention is given to what services or functions should be provided by cooperatives. There is interest in learning more about the attitude of members and nonmembers toward cooperative marketing, especially the differences in these attitudes.

In the last few years more attention is being given to the role of cooperatives in achieving bargaining power for farmers. In connection with market structure studies, special attention is being given to the impact of cooperatives on market conduct and performance.

The total research effort on cooperative marketing in the 10 States is 2.5 professional man years.

#### REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

##### A. Coordination of marketing

Farmers and their cooperatives need to adapt their marketing methods to the requirements of large-scale buyers, mass merchandising, and other changed conditions. In many cases the coordination of marketing of a number of cooperatives, marketing the produce of hundreds or thousands of farmers is needed to satisfy these needs and improve returns to farmers. Such coordination may be accomplished by establishment of joint sales agencies or by other methods. Research to determine the problems and needs, and to develop guides for adopting new practices, included work with several commodities.

1. Dairy. Cooperatives market about three-fifths of all milk marketed, either as milk or manufactured dairy products. With continued improvement in plant technology and transportation systems, markets are becoming less defined. During the past year, there was a marked increase in mergers of the major dairy cooperatives.



A study of the extent and types of coordination of marketing activities among dairy cooperatives is underway.

2. Poultry. Research was underway to determine the advisability of coordinating or combining all or part of the operations of three New England producer organizations.

B. Improving cooperative sales, distribution, and pricing methods

Wholesale and retail marketing practices are continuing to change rapidly, and there have been fundamental changes on the farm. For these reasons sales and distribution and pricing methods need to be studied carefully to plan and realize methods and policies that are technically efficient and obtain good returns for producers. Bargaining methods and pooling are two topics that require major research emphasis. Research on these problems included work in several commodities.

1. Bargaining (dairy, deciduous fruit and tree nuts, eggs, poultry, sugar beets, and vegetables). Research concerning bargaining methods and results was expanded to include dairy, egg, poultry, and sugar beet bargaining. This research seeks to appraise the status, role, and potentials of cooperative bargaining as a means of stabilizing and enhancing the incomes of producers.

2. Dairy. A study has been initiated that will examine the sales methods and distribution practices used by dairy cooperatives and the extent to which they coordinate their sales of manufactured products. The main objective will be to determine how they may better coordinate distribution of manufactured products to increase returns to farmers.

A study of pooling principles and practices was reactivated under a new project leader. This study will examine the effect of various pooling methods on equity among members and marketing efficiency in cooperatives subjected to changing marketing conditions.

Assistance was provided a newly organized federation of three cooperatives in developing procedures for pooling proceeds to members. The study indicated that the key to an acceptable pooling system was a full understanding of the marketing problems involved.

3. Poultry. Research was continued concerning opportunities and methods to improve fowl marketing by cooperatives and other handlers. Findings indicate that farm prices obtained by producers appear low in relation to utilization value of fowl. The fowl market appears to lack effective competition, particularly to get producers a share in the extra margins accruing from further processing of fowl. Cooperative effort by producers themselves establishing and operating slaughter and further-processing facilities for

fowl would potentially gain participating producers a direct share in improved returns, and through competitive effect of the cooperative, raise the price to other producers.

Research findings concerning pooling and producer payment practices of egg marketing cooperatives were being prepared for publication. Analysis included the relation of pricing differentials to cost factors, including size of shipments. It was found that cooperatives have seldom based pricing differentials on study of cost factors. Analysis of individual collection, plant, and office costs by each association would give it the basis for a system of equitable pricing differentials. Such differentials are effective in holding and attracting large producers.

4. Wool. A study conducted under contract with Iowa State University showed that in 1963, Iowa farmers marketed about 25 percent of their wool through cooperatives. The smaller growers showed more tendency to market their wool to feed dealers, hardware stores, and other local wool dealers than did the larger sheep producers. Many smaller sheepmen did not consider the value of their wool to be an important item of their farm income.

Results of a nationwide study of the operations of cooperative wool pools were published. The study concluded that pools enable growers to market their own wool more effectively and increase their net returns from wool by 3 to 4 cents a pound. Largely because of changes in the wool marketing system, growers with small farm and range flocks are showing new interest in this century-old form of marketing organization. The study led to suggestions for improving pool organization, assembly, grading, selling and management practices.

### C. Potentials in cooperative marketing

The present and potential role of cooperative marketing requires study in several commodity areas. Current information on cooperative operations can be related to production and marketing conditions. The objective of such research is to develop recommendations about operations and services of existing cooperatives and particularly to identify opportunities for farmers to increase their marketing returns by developing significant new areas of cooperative operation.

1. Dairy. A study of cooperative marketing activities and facilities is underway. This research will provide benchmark data on cooperative plant location, major equipment, and operating capacity. It will analyze the potential benefits to dairy farmers from increased coordination of their current activities or through other changes in organization and major methods of operation.

2. Livestock. Studies under contract with universities in Montana and North Dakota to determine the economic feasibility for cooperative feedyards and slaughter facilities were completed. These studies indicated that establishment of slaughter plants would not be as successful as feedlots because most areas now have excess slaughtering capacity. However, construction and operation of new, modern slaughtering facilities that are more efficient than the existing ones may be feasible. Securing farmers' full support of these undertakings is essential for operational success.

3. Poultry. Study of the operations and potentials of broiler auctions was continued. Preliminary findings indicate that while most broiler auctions have been discontinued, several have made substantial contributions to the pricing of broilers. Consideration is being given to the possibility of selling processed broilers at auction where market outlet conditions appear favorable.

The feasibility of a proposed cooperative turkey processing operation in the Midwest was analyzed and findings reported to grower leaders.

#### D. Improving operating and handling methods

Research was underway in several commodity fields to examine new methods, equipment, and structures for efficient and safe processing and storage of agricultural products by cooperatives.

1. Dairy. Dairy manufacturing cooperatives are caught up in the change to more diversified multiproduct operations. The managers of these operations must rely on cost and information systems to provide data on which to base decisions. A study has been initiated with the purpose of providing cooperative management with a standard departmentalized cost accounting system that will help them to control costs and improve efficiency and quality of service.

A case study was made of the operating practices and procedures of a fluid milk packaging cooperative that distributes its milk through wholesale and retail routes and through its own dairy stores. Findings indicate that the long-run interests of farmer-members were best served by the cooperative placing greater importance on selling its products through its own stores.

A system of product accounting was developed to improve the operating methods of a marketing federation.

A case study was made to determine the economic feasibility of a cooperative creamery adding a milk drying operation. Findings indicated that the patrons' best long-run interests may be served by the cooperative joining with other cooperatives in developing and utilizing large, efficient plant facilities.



2. Livestock. Work continued to analyze the alternatives available to livestock producers and cooperatives for integrating the production and marketing of livestock and meat. Preliminary results indicate opportunities for producers to benefit from maintaining control over their livestock through additional steps in the marketing chain. Several farmer groups and cooperatives were provided information to assist them in determining the feasibility of using such alternatives as feedlots or slaughtering and processing facilities.

Studies were made of four livestock cooperatives to assist them find ways to serve members better by improving operating efficiency, adding needed services, adopting new handling methods, or merging operations with another cooperative.

E. Improving the organization, financing, and management of marketing cooperatives

Studies were made to determine ways to improve the efficiency and assist cooperatives improve their services by analysis of organization, financing, and management practices.

1. Dairy. A study was made of the overall operation of a dairy bargaining cooperative. The analysis indicated that failure to make equitable charges for an expanded service program led to a decline in market position and bargaining strength.

2. Poultry. Study was continued of the organization and operations of a large, integrated regional marketing and supply cooperative. Preliminary findings include a case history of adjustments to meet changing conditions, and the relation of financial planning and conditions to changing operations.

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ECONOMIC AND STATISTICAL ANALYSIS  
Economic and Statistical Analysis Division, ERS

PROBLEM

Prices to producers are relatively unstable and conditions of agricultural production are changing rapidly. Thus, the farmer stands in special need of frequent accurate appraisals of his economic prospects if he is to plan and carry out his production and marketing activities in an efficient and profitable way. The typical farmer cannot afford to collect and analyze all the statistical and economic information necessary for making sound production and marketing decisions. It has long been a goal of the Department to provide the farmer with economic facts and interpretations comparable to those available to business and industry. Such information is provided through a continuous flow of current outlook information; the development of longer range projections of the economic prospects for the principal agricultural commodities; and analyses of the economic implications of existing and proposed programs affecting major farm commodities. The outlook program was strengthened during the year by extending the quarterly Outlook Digest to a monthly basis, to permit timely reporting of significant changes which may occur between issues of the various commodity Situation reports.

Producers, processors, distributors and consumers need more accurate quantitative knowledge of the interrelationships among prices, production, and consumption of farm products. Farmers and farm-related businesses need to know the prices they may expect from different levels of production to plan for maximum returns. Cooperatives, processors, and distributors need adequate statistical information on price and consumption responses under different supply conditions to aid in distribution of agricultural supplies that lead to maximum returns to farmers. Similarly, Congress and the administrators of farm programs need to evaluate alternative proposals to modify existing price support, production, marketing and other programs in terms of their impact on production, consumption and prices at both the farm and retail levels. The development of new statistical methods and the application of existing methods for measuring the interrelationships among prices, production, and consumption of farm products serve these needs by strengthening outlook and situation work, providing the basis for special analyses of alternative agricultural policies, and assisting research workers in agricultural economics.



## USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-range program of economic and statistical analysis directed in two major areas: (1) commodity situation and outlook analysis, and (2) supply, demand, and price of agricultural commodities.

The program pertaining to situation and outlook includes a continuous appraisal of the current and prospective economic situation of the major crop and livestock items. These appraisals, developments of interest to the industry, and results of special studies are published 4 to 6 times a year in the various commodity situation reports, with brief resumes in the quarterly Demand and Price Situation and the National Food Situation, and when appropriate in monthly issues of the Farm Index and the Agricultural Outlook Digest. Comprehensive analyses of the current and prospective situation are presented for each of the major commodities at the Annual Outlook Conference, and more limited appraisals given at regional and State conferences and at meetings with industry groups. Special analyses are prepared from time to time on the probable effect of proposed programs on the supply, price, and utilization of the various commodities. Basic statistical series are developed, maintained, improved, and published for general use in statistical and economic analysis. Statistical handbooks are published periodically for livestock and a number of field crops.

Except for a Regional Field Office for livestock in Denver, Colorado, all the USDA situation and outlook work is carried on from Washington. The regional livestock project is a cooperative effort including this division, the Federal Extension Service, and State Extension Services in the western states.

The professional man-years devoted to animal-poultry and product situation and outlook analysis amounts to 9.0 of which 4.5 are for livestock and meat (2.0 are in Denver); dairy, 2.0; poultry and eggs, 1.0; and wool 1.5.

The program of basic research into the factors affecting prices, supply, and consumption of principal agricultural commodities is concerned with four broad areas: (1) Measurement of consumer response to price, income, and other factors; (2) measurement of producer response to price and other factors; (3) measurement of the effect of supply and demand factors on prices to farmers and to consumers; and (4) improvement of statistical techniques for measuring agricultural economic relationships and for the development of statistical formulas which can be used in making price, supply, and consumption forecasts and in appraising economic implications of alternative programs.

Changes in emphasis are made from time to time to utilize effectively the professional skills available and to adjust to work having the highest priority. The research is commodity oriented, but in some areas such as livestock, livestock products, and feed, the research is part of an integrated analysis of the feed-livestock economy. The study of the beef cattle sector includes analyses of inventory numbers, of fed cattle marketings, of production and the composition of beef supply, and the impact of the growing importance of feedlots and emerging demand patterns for beef on the changing nature of the cattle cycle. Work in the livestock area also includes studies of the demand and price factors for eggs, broilers, and turkeys. Research on feed grains includes measurement of the influence of factors affecting supply and utilization among the different kinds of livestock with emphasis on substitution possibilities among these feed grains, and the extent to which they compete for the same farm resources. The current emphasis on cotton is to measure the economic factors that affect the price, supply, and utilization of cotton and cotton products, while that on tobacco is related to economic effects of technological changes including information relating to health on supply, demand, utilization, and price of leaf tobacco.

A facet that is becoming increasingly important in carrying out the statistical and econometric work of the Division is the use of electronic computers. The program includes continual evaluation of latest developments in the field, equipment and computer programs available for use, and the application of this to our data submitted for machine processing.

**The USDA program of research on supply, demand, and price of animal-poultry and products involves 9 professional man-years and is located in Washington, D.C. The effort is distributed as follows: livestock and meats, 2.0; and poultry and eggs, 2.5.**

#### PROGRAM OF STATE EXPERIMENT STATIONS

For the most part the States depend on the U.S. Department of Agriculture for the yearly across-the-board commodity situation and outlook research. There is increasing interest in longer range price prediction because of the growing specialization of farms, which makes yearly enterprise shifts less common and less feasible, and which calls for large capital commitments over longer periods of time. The State extension staff members supplement and adapt such research information to meet the commodity situation of their States.

The total direct research effort in the situation and outlook area is small--probably no more than 2 to 3 professional man-years. While not designated as outlook research, much of the research conducted by the experiment stations and reported elsewhere contributes to improved understanding of price-making forces, which in turn improves market situation analysis and price forecasting.



Many of the States carry on supply, demand, and price analyses for the products of their State. Much of the research is commodity oriented, though some projects are of a highly mathematical and theoretical nature aimed at improving price analysis methodology.

The research on demand for a large number of commodities will indicate the price elasticity, the income elasticity, and the cross elasticities of the commodities being studied. Because researchers are finding that some changes cannot be explained by price, income, and supply of competing commodities there is increasing research interest in social and psychological factors affecting demand.

The supply response to price changes is a matter that is receiving considerable attention. This is in part because of its significance to farm incomes and government programs. Significant progress is being made in **understanding the relationship of the capital structure on farms to supply response and thus to the difference between long-run and short-run supply responses.**

## PROGRESS - USDA AND COOPERATIVE PROGRAMS

### A. Commodity Situation and Outlook

#### 1. Livestock and Meat

In addition to the regular situation and outlook work, several special analyses were made during the past year. Additional consideration was given to the character of the present cattle cycle and how it has differed from earlier cycles. This work has centered on the nature of changes in the makeup of the cattle inventory and the relationship between cow slaughter and the beginning year cow inventory. Attention was also given to the relationship between number of cattle marketed from feedlots and the number on feed at the beginning of the quarter, by weight groups and by regions.

With higher cattle prices and lower average marketing weights that developed during the year, a study was made on price premiums for cattle of different weights in various market situations.

Attention has been focused on cattle productivity and some progress has been made in looking into this subject. Special work was done on feeding margins and their influence on returns on cattle feeding operations. A steer-composite feed price ratio was developed as a substitute for the steer-corn price ratio that has been used in evaluating probable effects on fed cattle output of changes in steer and feed prices. With hog prices up substantially this year, and the resulting quite favorable hog-corn price ratio an analysis was made of the possible increase in farrowings next year and the probable effect on farm price.



## 2. Dairy

Considerable effort has been devoted to analyzing the effects of existing and proposed Government programs as they affect milk production, marketings, consumption, stocks, and income. Other work in this area includes analyses of changes in imports and exports of dairy products, fluid milk pricing plans, and dairy farm income by value of sales classes.

An analysis of the role government plays in pricing fluid milk was made, showing the operations and methods used in Federal milk marketing orders, State milk control programs and State programs for regulating sales below costs. In all, milk sold under State and Federal marketing orders represents 90 percent of all milk that meets sanitary standards for fluid use. The report found that in addition to 76 Federal milk orders covering urban areas in all parts of 37 States, milk control agencies in 20 States fix prices. Seventeen of these latter States regulate various trade practices. Nine additional States have laws specifically regulating the sales of dairy products below cost or other trade practices.

Supplement for 1963-64 to Dairy Statistics, Statistical Bulletin No. 303, was issued in July 1965.

A simplified flow chart of milk production and utilization for 1964 was published in the September 1965 Dairy Situation with comments, updating material published in 1957 in USDA Technical Bulletin 1168.

## 3. Poultry and Eggs

Several topics received special attention during the past year. The May issue of the Poultry and Egg Situation featured an analysis of changes in the hatchery industry. Hatcheries in recent years have become larger, fewer in number, and more closely integrated with other segments of the poultry industry. These changes, together with reduced seasonality of production in the case of chicks, have contributed to a substantial reduction in excess capacity. It was estimated that chick hatcheries in 1964 utilized about 60 percent of their egg setting capacity compared with only 40 percent in 1957. Turkey hatcheries in 1964 utilized only about a third of their capacity--due partly to the highly seasonal nature of poult hatchings. The July Situation focused attention on the large cyclical buildup that was getting underway in broiler production and compared the factors operating in the current expansion with those in past expansions. Producers were warned that if this buildup continued they would probably experience a long period of depressed prices more severe than in 1963-64. The September issue included a discussion of the factors in the declining demand for eggs in recent years and concluded that the downtrend may be continuing. A statistical supplement to the Poultry and Egg Situation was issued for the first time in May 1965.

#### 4. Wool

A number of special studies were made during the year. Special analyses related to foreign trade in raw wool and wool textile products and their impact on the U.S. wool industry. Studies also were made for the Secretary's office on the impact of various proposed changes in the wool program on production of wool, incentive payments, and prices received by growers. Quarterly estimates relating to wool were provided to the Commonwealth Economic Committee.

The data include estimated mill use of raw wool and all other fibers in the woolen and worsted industry, production of wool tops, yarn, and fabric and stocks of raw wool and tops. In addition, forecasts are made for mill use of apparel and carpet wool for the next quarter.

### B. Supply, Demand and Price Analysis

#### I. Livestock and Meat

Congress passed meat import quota legislation (Public Law 88-482) in August 1964 that required the Secretary of Agriculture to make estimates of domestic production of beef, veal, mutton, and goat meat for the coming calendar year each preceding December as part of the procedure for determining whether an import quota should be imposed during that year. Special analyses of factors affecting the production of these meats were made in late 1964 to provide a statistical basis for forecasting the production of beef, veal, mutton and goat meat for calendar year 1965. Further work is being done in 1965 to refine the models used in these analyses for the projections that will be made for 1966.

A beef cattle inventory study was completed and published. Based on historical relationships between cattle numbers and past prices, formulas were constructed in this study which could be used to forecast the numbers in each of four classes of cattle and calves from the latest price information available. These forecasts can be made from six months to more than a year in advance. For example, inventory numbers on January 1, 1967, could be forecast from information available in late summer of 1965. The individual forecasts of each class of cattle can be combined to form a forecast of the total beef cattle inventory. This study indicated that beef cow and calf numbers would drop when feeder calf prices fall and stay below \$21.00 for a sustained period of time.

A supply response model was developed which considers fed beef and non-fed beef slaughter as two separate components of beef supply. New long-time series for fed and non-fed beef had to be developed for use as data in this model. Preliminary results from this model for fed and non-fed beef indicate that the price margin between steer and feeder calves,



the steer-corn price ratio, and the number of cattle available for feeding were important factors in determining the number of fed beef animals slaughtered. Parts of this study along with the new data developed will be published shortly.

Some progress has been made toward testing different series which could be a better measure of feed costs for cattle in place of the currently used steer-corn price ratio. A description of how total returns to fed cattle can be broken down into returns to feeding and returns to margins has been completed.

Some progress has also been made in the study begun in 1964 to improve quarterly forecasts of marketings and prices of fed cattle, prices of feeder animals, and the number of animals placed on feed. However, more work is needed in this area to develop statistically acceptable formulas for adequate forecasting in situation and outlook work, particularly the formula for the number of animals placed on feed.

As part of a continuing program of evaluating previously developed statistical models, the quarterly supply model developed in 1962 for determining the number of hogs farrowed and slaughtered and the price of hogs was tested for its ability to make current forecasts and to determine ways to adapt the model to recent changes in cyclical and seasonal patterns of hog production and prices.

## 2. Poultry and Eggs

Research was initiated to identify and statistically measure the impact of factors influencing the supply, demand, and price of eggs. Analyses are being developed for the United States as a whole and for individual regions. Preliminary findings to date indicate that changes in egg supplies and a downtrend in egg demand explain most of the variation in egg prices over the last decade. Analyses also show that egg prices in recent years have declined most in States and regions moving from a deficit to a surplus position in egg production.

Turkey demand and factors influencing turkey prices, in and outside the heavy fall marketing period, were analyzed for the first time, using new data which have become available only recently. Major findings of the study include: (1) The quantities of turkey consumed is much more responsive to changes in the turkey price in the January-August period than in September-December period; (2) farm turkey prices in January-August are affected by the current level of chicken supplies but prices in September-December are not; and (3) farm turkey prices in September-December are influenced by the year-to-year change in poultry consumption in the preceding 8 months. The price predicting equations developed gave estimates of the farm turkey price in September-December and for the year



as a whole that were within 1 cent or less of the observed price for each of the 10 years in the study. Estimated prices for the January-August period were within 1 cent of observed prices in 7 out of the 10 years, the greatest deviation being 1.7 cents. Results of this study are expected to be ready for publication in the next few months.

## PUBLICATIONS - USDA AND COOPERATIVE PROGRAMS

### Commodity Situation and Outlook Analysis

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**CONSUMER PREFERENCE AND QUALITY DISCRIMINATION--  
HOUSEHOLD AND INDUSTRIAL  
Standards and Research Division, SRS**

Problem. With the increasing complexity of marketing channels and methods, it has become almost impossible for consumers to express to producers either pleasure or displeasure with available merchandise. To market agricultural products more effectively, it is necessary to understand existing household, institutional, and industrial markets and the reasons behind consumers' decisions to purchase or not to purchase. Information is needed on consumers' attitudes toward old and new product forms of agricultural commodities, preferences, levels of information or misinformation, satisfactions or dislikes, and what product characteristics would better satisfy current consumers and/or attract new ones. It is also important to know the relationship between the consumption of one agricultural commodity and another in consumers' patterns of use, the relationship between agricultural and nonagricultural products, and probable trends in the consumption of farm products. Producer and industry groups as well as marketing agencies consider such information essential in planning programs to maintain and expand markets for agricultural commodities which, in turn, increases returns to growers.

**USDA PROGRAM**

The Special Surveys Branch conducts applied research among representative samples of industrial, institutional, or household consumers and potential consumers. Such research may be conducted to determine preferences, opinions, buying practices, and use habits with respect to various agricultural commodities; the role of competitive products; acceptance of new or improved products; and consumers' ability to discriminate among selected attributes of a product or levels of an attribute, and the preferences associated with discriminable forms.

In addition to the studies of consumer preference and discrimination, the Branch also provides consultants and conducts special studies, upon request, for other agencies in the USDA or within the Federal Government, when survey methods can be usefully applied to the evaluation of programs, services, or regulatory procedures of interest to the requesting agencies.

The research is carried out in cooperation with other USDA or federal agencies, State experiment stations, departments of agriculture, and land-grant colleges, and agricultural producer, processor, and distributor groups. Closely supervised contracts with private research firms are used for nationwide surveys; studies in selected areas are usually conducted by the Washington staff with the assistance of locally recruited personnel.



The Branch maintains all of its research scientists, who are trained in social psychology or other social sciences, in Washington, D. C., which is headquarters for all the research whether it is conducted under contract or directly by the Branch. The Federal scientific effort devoted to research in this area during the past year totaled 7.0 professional man-years. An additional .1 professional man-year was devoted to research conducted under a transfer of funds arrangement.

## REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

### A. Consumer Preference

1. Fibers in wearing apparel. The rapid expansion in recent years in the use of manmade fibers and blends necessitates up-to-date evaluations of consumer reactions to natural fibers in specified end uses. Such data give industry a better understanding of its markets, and provide a guide for planning physical science research in industry and within the Department on product improvement as well as educational, promotional, and merchandising efforts designed to strengthen the market position of cotton and wool.

A preliminary report on the results of a contract study on reactions to fibers in selected items of clothing among a nationwide sample of teenage boys and girls was issued during the fall of 1964; a final report presenting more detailed findings is in preparation. Data from the study have been discussed in a previous progress report.

The exploratory phase of the nationwide contract study of women's opinions about fibers in apparel for warmer weather was completed. In the exploratory interviewing, groups of women were studied in depth to determine their attitudes and opinions with respect to certain items of clothing and the fibers in them. The findings guided the designing of two types of questionnaires for use among comparable samples of women. This will permit detailed evaluation of the advantages and disadvantages of the two methods. One of the questionnaires is composed mainly of structured questions; the other uses the traditional open-end questions to collect the attitudinal responses. The pretest for this study is scheduled for July; actual field work is planned for early fall of 1965.

2. Poultry. A nationwide survey is being conducted under contract by a private market research firm to ascertain household consumers' preferences, use patterns, and purchasing practices for broilers-fryers and turkeys. Data from the study will provide insights into the measures which might be taken by the poultry industry to market their products more effectively and to increase demand among consumers. To supply information about trends in preferences and attitudes among representative consumers, some questions were replicated from an earlier (1956) study. The data will also be of value to market specialists and technologists in the Department who are interested in doing further developmental work on convenience items, or in shaping new markets for products having marginal characteristics. A

preliminary report on the study has been issued. Additional data analysis and preparation of the final report are underway. According to the preliminary analyses, the proportion of homemakers reporting usage of broilers or fryers has increased since 1956 from 93 to 97 percent. Frequency of usage has also increased; 64 percent of the users in 1964 reported serving broiler-fryers once a week or more compared to 50 percent in 1956. Homemakers with larger families tended to use broiler-fryers more often. Most homemakers reported being able to purchase broiler-fryers in the weight range which suited their family's needs. Purchasers were asked to choose from a list of specified items those characteristics which they felt were most important in selecting broilers or fryers. About 70 percent chose "one that is well cleaned," "no bruises or discoloration," and "the right size." About 60 percent selected "inspected by the government," and a little over 50 percent selected "no pinfeathers," and "plumpness." About 40 percent said they "depend on the store" while only 20 percent indicated that the brand name was important in their selection process.

When asked which of three poultry grade labels--words, letters, or numbers--would be the easiest to understand, almost 60 percent selected words, while 30 percent chose letters. Only 5 percent felt that numbers would be easier to understand.

The results of the two surveys turned up some changes during the last decade in the ways homemakers prepared chicken for their families. Frying was the most popular method in both surveys. But its popularity has declined slightly, while increases were reported for other methods such as baked or roasted, broiled, and barbecued.

Outdoor cooking increased considerably. In the 1964 study, 35 percent of the homemakers said they had cooked broiler-fryers outdoors. Only 11 percent reported doing so in 1956.

The proportion reporting use of turkey (76 percent) increased by 11 percent since 1956, although turkey is still served rather infrequently. About the same proportion of users (3 out of 4) in both surveys reported a rate of serving of only 1 to 3 times during the year.

3. Milk. The study of milk consumption patterns in Milwaukee, Wisconsin, and New Orleans, Louisiana, with particular emphasis on low-fat (sometimes called two percent milk) has been completed. The final report on this study, which was conducted in cooperation with ERS, was published in May 1965.

In Milwaukee, where low-fat milk has been available since 1951, about 15 percent of the families interviewed had used low-fat milk within six months prior to the survey. In New Orleans, where low-fat milk has been available only since 1957, about 6 percent of the families had used low-fat milk within six months of the interview. Low-fat milk makes its primary appeal to diet-conscious customers. More than 8 out of 10 of the families using low-fat milk had a dieter or weight watcher in the household. Low-fat milk is also more apt to be tried by the relatively high-income household.



The majority of the families using low-fat milk in both cities were satisfied with the price. However, a somewhat higher proportion of families in New Orleans thought the price they paid was too high. In New Orleans, low-fat milk was not only more expensive than it was in Milwaukee, but it also cost more than whole milk.

A contract (financed in part by ARS) has been signed with a local university research group to conduct a survey to evaluate consumer acceptance of powdered dry whole milk, a new product developed by the Dairy Products Laboratory of the Eastern Utilization Research and Development Division, ARS. At this time, the product compares favorably with fresh whole milk when tested under controlled conditions (see Quality Discrimination Section), and looks commercially feasible for distribution under refrigeration. However, the reaction of the housewife to the reconstitution behavior of the product and the residual foam on the surface of the reconstituted milk are untested and unknown. Evaluation of the product by consumers under natural conditions is, therefore, felt to be desirable not only to mark the extent of present progress but also to indicate lines along which further research on the product should proceed. The field work on this study is planned for the early fall of 1965.

#### B. Quality Discrimination

The sensory testing laboratory of the Branch is used to ascertain, under controlled conditions, people's abilities to discriminate among qualities or levels of a quality for food samples, or other sensory or visual stimuli, and the preferences associated with discriminable variables. The products which have been evaluated include new food forms developed in the ARS laboratories or variations of products already available. Studies have been conducted this year on apple juice, milk, and an apple-grapefruit juice blend. Studies on Hawaiian coffee are in progress and research on grapes is planned. Some examples of the types of problems investigated are listed below. The results of these studies have not been published, but were reported by memorandum to the cooperating group requesting the research.

Milk. Three commercial products (fresh whole milk and fluid and dry nonfat milk) were procured on the market place for comparison with an experimental dried whole milk developed by the Eastern Utilization Research and Development Division, ARS. The nature of the comparison was preference. Results of the study indicated that the experimental dried whole milk was as well liked as fresh whole milk, and that the experimental product was preferred to the other two commercial products.



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